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CLINICAL REPORT

A monoallelic *SEC23A* variant E599K associated with cranio-lenticulo-sutural dysplasia

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Abstract

Cranio-lenticulo-sutural dysplasia (CLSD; MIM 607812) is a rare or underdiagnosed condition, as only two families have been reported. The original family (Boyadjiev et al., *Human Genetics*, 2003, 113, 1–9 and Boyadjiev et al., *Nature Genetics*, 2006, 38, 1192–1197) showed recessive inheritance of the condition with a biallelic *SEC23A* missense variant in affected individuals. In contrast, another child with sporadic CLSD had a monoallelic *SEC23A* variant inherited from the reportedly unaffected father (Boyadjiev et al., *Clinical Genetics*, 2011, 80, 169–176), raising questions on possible digenism. Here, we report a 2-month-old boy seen because of large fontanels with wide cranial sutures, a large forehead, hypertelorism, a thin nose, a high arched palate, and micrognathia. His mother was clinically unremarkable, while his father had a history of large fontanels in infancy who had closed only around age 10 years; he also had a large forehead, hypertelorism, a thin, beaked nose and was operated for bilateral glaucoma with exfoliation of the lens capsule. Trio genome sequencing and familial segregation revealed a monoallelic c.1795G > A transition in *SEC23A* that was de novo in the father and transmitted to the proband. The variant predicts a nonconservative substitution (p.E599K) in an ultra-conserved residue that is seen in 3D models of yeast SEC23 to be involved in direct binding between SEC23 and SAR1 subunits of the coat protein complex II coat. This observation confirms the link between *SEC23A* variants and CLSD but suggests that in addition to the recessive inheritance described in the original family, *SEC23A* variants may result in dominant inheritance of CLSD, possibly by a dominant-negative disruptive effect on the SEC23 multimer.

KEYWORDS
coat protein complex II, cranio-lenticulo-sutural dysplasia, SEC23A, whole genome sequencing

1 | INTRODUCTION

Cranio-lenticulo-sutural dysplasia syndrome (CLSD; MIM 607812) was originally described in a large consanguineous Saudi Arabian family with six affected individuals. In that family, the disorder was characterized by late-closing fontanels, frontal bossing, macrocephaly, hypertelorism, a broad prominent nose, and short stature. In addition, several patients had an ocular phenotype of Y-shaped sutural cataracts, "double-ring" sign in the eyes and/or optic nerve hypoplasia (Boyadjiev et al., 2003, 2006, 2011). Intellectual development was not affected. Linkage analysis mapped the disease locus to chromosome 14q13-q21 (Boyadjiev et al., 2003, 2006). Subsequently, direct sequencing of candidate genes in the linked region revealed a homozygous variant in *SEC23A* (NM_006364.4: exon10; c.1144 T > C; p.F382L), segregating with the phenotype in an autosomal recessive fashion.

Since the original reports of this family, only one additional unrelated patient was reported as having CLSD. In this patient, a single heterozygous missense variant in *SEC23A* (NM_006364.4: exon19; c.2104A > G; p.M702V) was identified; however, this variant was present also in the reportedly unaffected father, leading to the hypothesis of digenic inheritance of CLSD (Boyadjiev et al., 2011). More recently, Kim and Boyadjiev have suggested that for this specific variant a dominant mode inheritance with incomplete penetrance was also a possible alternative to recessive or digenic inheritance (Kim & Boyadjiev, 2016).

SEC23A encodes for one of the main components of a protein coat complex called coat protein complex II (COPII) that is responsible for the generation of transport vesicles that export most cargo molecules from the endoplasmic reticulum (ER) toward the Golgi complex for secretion (Mancias & Goldberg, 2008). COPII consists of five cytosolic proteins: SAR1, SEC23, SEC24, SEC13 and SEC31 (Tang et al., 2005). COPII formation is a dynamic process initiated by the activation of small GTPase SAR1 catalyzed by SEC12 (Figure 2b, step I.). Membrane bound SAR1-GTP recruits SEC23-SEC24, through the interactions with the SEC23 subunit to form the prebudding complex SAR1-SEC23-SEC24 (Figure 2b, step II.) (Yoshihisa et al., 1993). This complex recognizes signals from the SNARE and cargo molecules, and finally the recruitment of SEC13-SEC31 complex, promotes coat polymerization and membrane deformation in order to form the transport vesicles (Figure 2b, step III.). In addition, SEC23 acts as a GTPase-activating protein (GAP) for SAR1, and SEC23 mediated hydrolysis to SAR1-GDP, is further stimulated by SEC13-31. SAR1-GDP has much lower affinity for SEC23-SEC24-SEC13-SEC31, leading to coat disassembly after completion of polymerization and vesicle formation (Figure 2b, step IV.) (Antonny et al., 2001).

In the human genome, there are two paralogs, *SEC23A* and *SEC23B*, which have a sequence identity of 84.5% and share many identical regions. They are both ubiquitously expressed but with variable ratios, which likely leads to their different functions as well as the difference in phenotypes they are associated with (Khoriaty et al., 2018). Indeed, variants in *SEC23B* lead to congenital dyserythropoietic anemia type II (MIM 224100), a disease with no craniofacial or skeletal defects (Bianchi et al., 2009). Two different pathogenic mechanisms have been invoked for the two *SEC23A* variants associated with CLDS so far, based on limited experimental evidence. The homozygous p.F382L *SEC23A* mutant directly weakens the binding between SEC13-31 and SEC23-SEC24 complex, leading to the inhibition of vesicle formation. This in turn results in an accumulation of secretory material within the ER of patients' fibroblasts (Boyadjiev et al., 2006; Hughes & Stephens, 2008). The p.M702V variant in *SEC23A* has been found to affect specifically the exit of procollagen from the ER but not that of other cargo molecules. This variant appears to accelerate the rate of SAR1-GTP to SAR1-GDP conversion and does not impact the binding between SEC23 and SEC13-SEC31. The quicker rate of SAR1-GTP hydrolysis may cause a premature disassembly and detachment of COPII coat from the ER membrane, likely leading to the exclusion of larger cargo molecules, such as procollagen, from the forming vesicle (Boyadjiev et al., 2011; Erickson & Wynshaw-Boris, 2016). However, there is little phenotypic overlap between CLSD and the type 1 or type 2 collagen disorders, suggesting that additional pathogenic mechanisms are likely to exist.

Here, we report on a father and son who both had features of CLSD and a monoallelic variant affecting an ultra-conserved residue of SEC23A situated at the interface between SEC23A and the SAR1 protein, suggesting that dominant inheritance of CLSD may occur.

2 | METHODS

The proband's parents signed an informed consent form for molecular studies to reach a diagnosis of an undiagnosed disorder of craniofacial morphology. Subsequently, the boy's paternal grandparents gave informed consent for the testing of the *SEC23A* variant identified in the family in their DNA samples. The boy's parents gave explicit consent to the publication of their photographs and clinical data and the molecular data concerning *SEC23A*.

Genome sequencing was performed on the proband and his parents. Genomic DNA was extracted from peripheral blood leukocytes according to standard procedures, and then sequenced on an Illumina Nova-Seq instrument by Novogene, UK. Novoalign software (V3.08.00, Novocraft Technologies) was used to map the raw reads to the human reference genome (hg19/GRCh37) and duplicate reads were removed using Picard (version 2.14.0-SNAPSHOT) (Picard, n.d.). Single nucleotide variants and small insertions and deletions (indels) were detected using the Genome Analysis Tool Kit (GATK v4.0) software package, following the Best Practice Guidelines (DePristo et al., 2011). Copy number variants and larger structural variants were detected using Parliament2 (Zarate et al., 2020) and merged using Survivor (Jeffares et al., 2017). All the single nucleotide variants (SNVs) and indels were annotated with ANNOVAR (Wang et al., 2010) in combination with in-house scripts and databases. AnnotSV (Geoffroy et al., 2021) was used to annotate structural variants calls. The impact of variants on splicing was predicted using SpliceAI (Jaganathan et al., 2019) and the probability of 5'UTR variants to perturb upstream open reading frame of *SEC23A* was evaluated using UTRannotator (Zhang et al., 2021), plugin to the Ensembl Variant Effect Predictor (Zhang et al., 2021). Variant annotations were verified with VariantValidator (Freeman et al., 2018).

The presence of p.E599K *SEC23A* variant (NM_006364.4: exon 16; c.1795G > A) was confirmed in the proband and his father by Sanger sequencing, which was also used to test the presence of the variant in the proband's paternal grandparents.

A model of the SEC23-SAR1 from *Saccharomyces cerevisiae* (1M2O; Bi et al., 2002; Freeman et al., 2018) was downloaded from PDB (version April, 2021) (Berman et al., 2002), and the 3D model of the structure was visualized using ChimeraX (Pettersen et al., 2021).

3 | RESULTS

3.1 | Clinical descriptions

A boy was referred to Clinical Genetics at age 2 months with the suspicion of a genetic syndrome. Prenatal ultrasound had suggested moderate shortening of humeri and femurs. On morphological ultrasound performed at 20 weeks, fetal anatomy was normal, fetal growth appeared regular while femur length was at the 5th percentile for gestational age. Length of the bones was as follows: femur 29.5 mm (5th percentile), humerus 29.2 mm (10th percentile), foot 32 mm (the ratio femur/foot was 0.92), scapula 16 mm (50th percentile), radius 25 mm (25th percentile), ulna 28 mm (25th percentile), tibia 27 mm (25th–50th percentile), and fibula 26 mm (25th percentile). Head circumference was 176 mm (25th–50th percentile) and abdominal circumference 154 mm (50th percentile). On subsequent ultrasound scans, performed at 34 weeks and 37 weeks of gestational age, fetal biometry was globally below the 5th percentile, suggesting a generalized growth disturbance. He was born by C-section at week 39 because of abnormal cardiotocography (CTG) findings. Weight was 2462 g (<3rd percentile −2.19 SD), length 47 cm (3rd to 10th percentile −1.7 SD), head circumference was 31 cm (<3rd percentile −2.84 SD), according to the WHO growth charts (WHO Multicentre Growth Reference Study Group, 2006). Apgar 1':9, 5':10. Facial dysmorphism was noticed and large cranial defects were palpated. An array CGH was done that gave normal results. Cleidocranial dysplasia was suspected because of the large cranial defects; radiographs confirmed the wide sutures, but the clavicles were normal. When seen at age 2 months, there were large fontanels (>8 cm wide) with wide cranial sutures, a large forehead, hypertelorism with outer canthal distance of 8 cm (>97th percentile), inner canthal distance of 3 cm (>97th percentile), and interpupillary distance of 5.5 cm (>97th percentile), slightly downward sloping palpebral fissures, a thin nose, a high arched palate, and micrognathia (Figure 1). Length was 52 cm (<1st percentile, −2.59

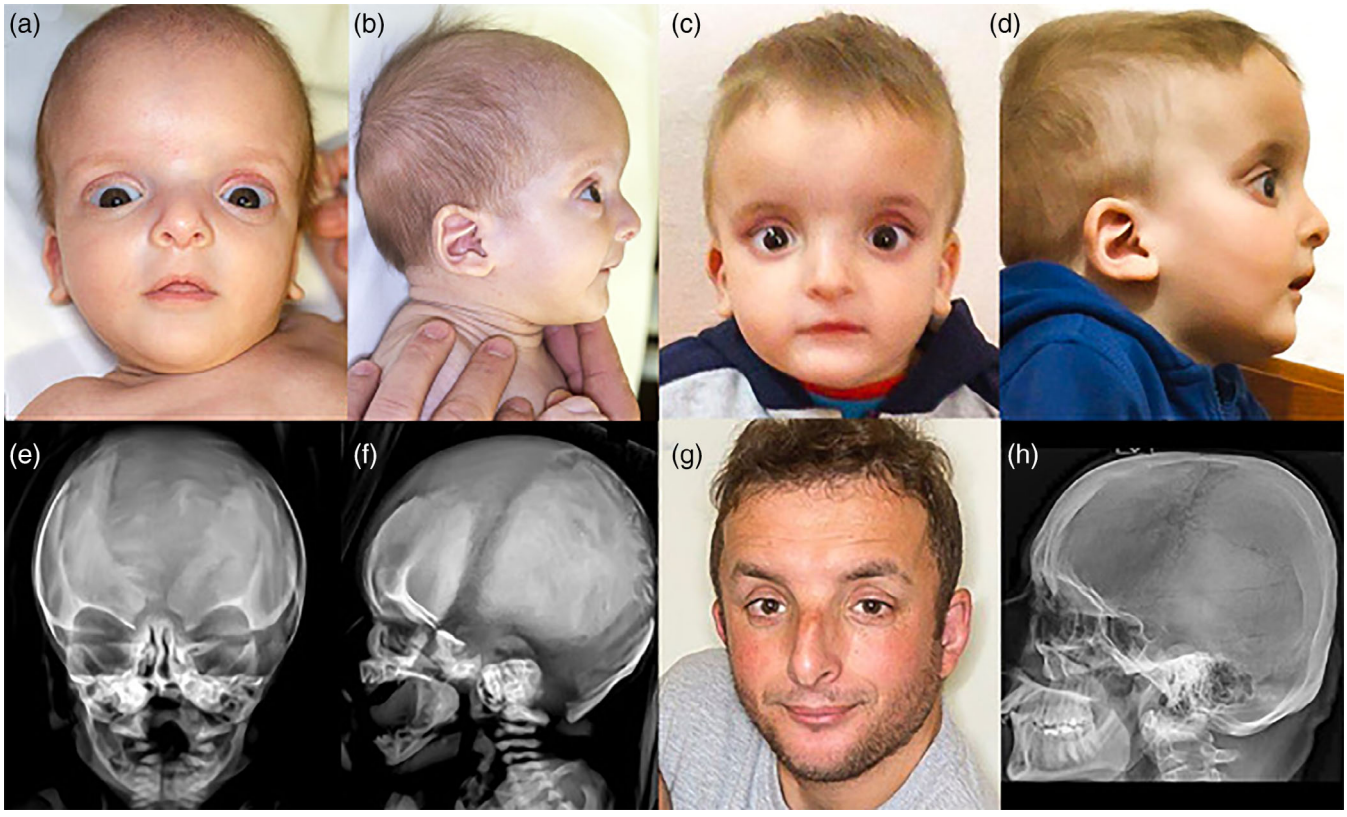

FIGURE 1 (a–d) Facial phenotype of the proband at age 2 month (a,b) and at age 18 months (c,d). Note large forehead, hypertelorism, downslanting palpebral fissures with "sunset phenomenon" (panel b) and thin nose with hypoplastic alae nasi. There is a striking resemblance to the individuals reported by Boyadjiev et al. (2003, 2006, 2011). Panels (e) and (f) show the skull radiographs of the proband at birth. Note the very incomplete ossification of the frontal, parietal, and occipital bones leaving large sagittal and coronal sutures (lambdoid suture not well visible). Panel (g) shows the proband's father as an adult. His facial features include the large forehead, hypertelorism and thin nose. In his lateral skull radiograph (panel h), the fontanels are close but the frontal sinuses have failed to develop

SD), weight was 3990 g (3rd percentile, −1.83 SD), and head circumference was 37.5 cm (8th percentile, −1.37 SD) according to WHO growth charts (WHO Multicentre Growth Reference Study Group, 2006). When seen in follow-up at age 18 months, his anterior fontanel was still several centimeters wide and the sagittal suture was open; length, weight and head circumference had recovered and were just within normal limits (length was 78 cm, 12th percentile, −1.18 SD, weight was 10.2 kg, 10th percentile, −1.3 SD and head circumference was 47 cm, 29th percentile, −0.55 SD). His psychomotor development was normal. He reached autonomous walking at 15 months, spoke his first word at 16 months and several words at age 18 months. Teeth erupted at age 6 months and were morphologically normal. An ophthalmological evaluation at 18 months was within normal limits.

The boy's mother was clinically unremarkable. The father had a noticeable facial phenotype with a large forehead, hypertelorism characterized by outer canthal distance of 10 cm (>97th percentile), inner canthal distance of 4 cm (>97th percentile) and interpupillary distance of 7 cm (>97th percentile), and a thin, beaked nose (Figure 1). Importantly, he offered a history of large fontanels in infancy and childhood who had closed only around age 10 years and his medical charts indicated that he had had eye surgery for bilateral glaucoma. Bilateral embryotoxon and exfoliation of the lens capsule had been noted at surgery, and possible diagnoses of Rieger anomaly or of Alagille syndrome had been considered, but complete blood count (CBC), liver markers, and cholestasis markers in blood were normal. After the Yag-laser iridotomy, his ophthalmologic status and his visual acuity were normal. His stature was 163 cm, (3rd percentile, −1.89 SD) and his head circumference was 53.5 cm (14th percentile, −1.08 SD). His teeth were normal and they erupted at 6 months of life. Orthopantomography done at 29 years showed no alterations. He had mild learning disability and he completed compulsory education only. However, he is fully functional as an adult. His skull X-rays showed that the fontanels and sutures were closed but there was lack of development of the frontal sinuses. Both paternal grandparents were seen and examined; both were taller than their son, and their facial appearance was unremarkable.

3.2 | Molecular analysis

Trio genome analysis identified a monoallelic variant in the *SEC23A* gene, NM_006364.4: exon 16; c.1795G > A; p.E599K, present in the proband and his affected father, and absent in the clinically unaffected mother. Sanger sequencing of the probands' paternal grandparents showed that the variant was absent in both and had thus appeared de novo in the proband's father.

The c.1795G > A (p.E599K) *SEC23A* variant affected an ultra-conserved residue (Genomic Evolutionary Rate Profiling [GERP++] score of 5.49) and was predicted to be highly deleterious by several scoring algorithms (SIFT: Deleterious; Polyphen:Deleterious; CADD:32; REVEL:0.99; VEST4:0.918). Since *SEC23A* variants associated with CLSD have been previously reported to be recessive, we manually screened all the coding regions of the gene but did not identify any other protein impacting variant present in both the affected son and his father. The analysis of shared variants in the noncoding regions of *SEC23A* as well as of the downstream and upstream regions revealed a deep intronic variant: NM_006364.4: exon1: c.204 + 18942G > A, predicted to have no impact gene splicing as well as a 5'UTR variant that was predicted to not neither create nor disrupt upstream open reading frames. Both variants were transmitted from the father to the child, indicating they are all in *cis*.

In 2011, Boyadjiev et al. (Boyadjiev et al., 2011) observed a boy with CLSD who had a single heterozygous *SEC23A* variant. Because the original CLSD family was consistent with biallelic recessive inheritance, they hypothesized that CLSD could be a result of a combined action of two (or more) variants in different genes and thus that the disease would follow a digenic (or oligogenic) mode of inheritance. To test the hypothesis of digenism, we extracted from the genome data all the variants shared by the affected father and son and absent in the unaffected mother. We then excluded variants with a minor allelic frequency (MAF) above 0.01 as well as those with no impact on the encoded protein, retaining only missense and loss of function variants, or intronic variants predicted by SPliceAI to have an impact on the splicing. In these retained variants, we searched for those who would be in a gene coding for a protein known to interact with SEC23A as determined by the STRING database of protein–protein interactions (SAR1A, SAR1B, SEC13, SEC16A, SEC22B, SEC24A, SEC24B, SEC24C, SEC24D, and SEC31A) (Jensen et al., 2009). None of these variants was in a gene known to interact with SEC23A.

To evaluate the potential effect of the p.E599K variant on the SEC23A structure, we examined the structure of the COPII complex in yeast, in particular at the structure of the SEC23A functional homolog in yeast, SEC23. Glutamate in position 599 corresponds to glutamate in position 605 in yeast SEC23. p.E605 is one of the 24 ultra-conserved residues contacting SAR1. Notably, together with the invariant residues FNNS in positions 599–602, that are in the αM-αN loops of the helical domain of SEC23, that forms the interaction patch I between SEC23 and residues WHP (55-56-57) in SAR1. These residues are the most critical for the recognition of SAR1-GTP (Bi et al., 2002).

4 | DISCUSSION

Agnostic trio genome analysis of father and son with a disorder affecting the skull and facial appearance and the unaffected mother revealed, as the most outstanding finding, a monoallelic missense variant in *SEC23A* never seen previously in public databases. Segregation analysis indicated that the *SEC23A* had appeared de novo in the affected father and transmitted from him to the affected child. Bioinformatic analysis of the genome data suggested that there were no other potentially pathogenic variants in *SEC23A*, nor in any of the genes coding for SEC23A-interacting proteins, making recessive and digenic inheritance unlikely. Thus, the data suggested that the CLSD phenotype in this family was associated with a monoallelic, apparently

dominant *SEC23A* variant. The craniofacial and ocular phenotype in this family was similar to that of the original CDLS description, with the exception of the absence (so far) of ocular findings in the boy, and the stature of father and son that is at the lower limit of normal, albeit shorter than the family context. Radiographic studies failed to reveal signs of chondrodysplasia.

The *SEC23A* variant, p.E599K, affects a highly conserved residue and is predicted to be deleterious. 3D modeling shows that the affected residue is situated at the interface between SEC23A and SAR1. Interestingly, the two previously reported *SEC23A* variants causing CLSD, p.F382L and p.M702V, have both been found in SEC23A regions interacting with SEC31 instead (Boyadjiev et al., 2011; Fromme et al., 2007) (Figure 2a). It is thus plausible (though not proven) that the mutant SEC23A protein may have a negative effect on the assembly and the function of the COPII complex, and that this may explain its dominant nature. Functional studies to validate the assumption of a dominant negative effect of this variant are desirable but beyond the scope of this clinical-laboratory observation. Moreover, the absence of cataracts in the child and the absence of signs of skeletal dysplasia may suggest that CLSD in this family has a clinically milder expression than that originally observed in the family with biallelic variants. Thus, we conclude that this family provides evidence for dominantly inherited CLSD and we suggest scrutinizing heterozygous *SEC23A* variants for possible phenotypic effects rather than to discard them in the absence of a second variant. Further observations are needed to define the clinical spectrum and variability of CLSD in relationship with monoallelic or biallelic variants.

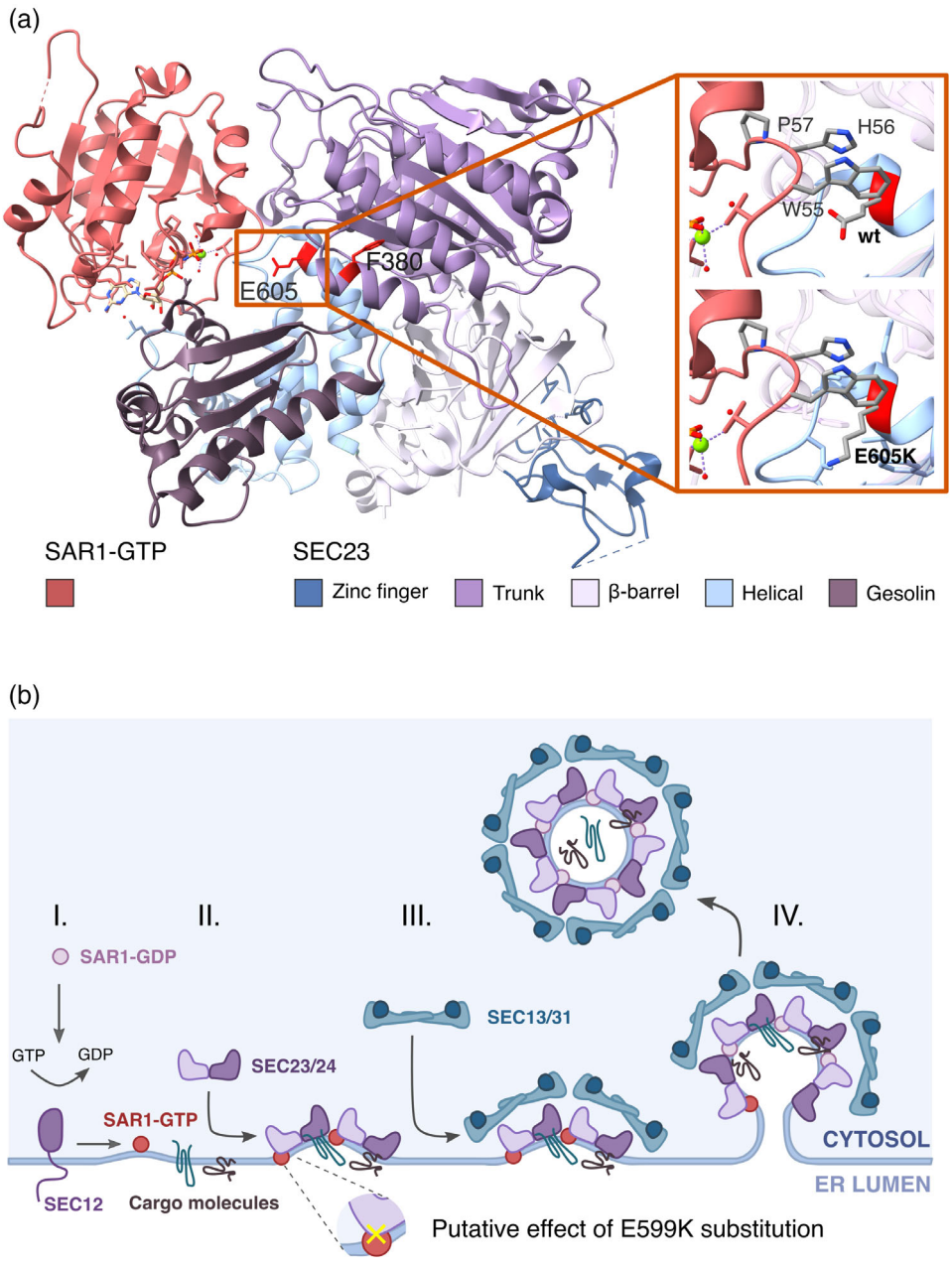

FIGURE 2 (a) Model of the SEC23-SAR1 from *S. cerevisiae*, showing the interaction of different SEC23 domains with the structure of SAR1. Positions of the reported p.F382L (corresponding to p.F380 in *S. cerevisiae*) as well as the here-described p.E599K (corresponding to p.E605 in *S. cerevisiae*) variants are highlighted in red. The variant p.M702V is not shown as its position is not conserved between *S. cerevisiae* and *Homo sapiens*. The structural details of the p.E605 and the variant p.E605K are also shown. (b) Steps of the COPII vesicle formation: I. Vesicle formation starts by GDP–GTP exchange on SAR1 catalyzed by SEC12. II. Activated Sar1-GTP then binds to the ER membrane and recruits the SEC23-24 complex through the interactions between SAR1 and SEC23. Cargo molecules are captured by direct contact with SEC24. III. The prebudding complexes are clustered by SEC13-31, and the binding of this subunit also promotes the hydrolysis of SAR1-GTP into SAR1-GDP and the dissociation of the forming COPII coated vesicle from the membrane (IV.)

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Conceptualization and planning: Andrea Superti-Furga and Livia Garavelli; clinical data collection and interpretation: Francesca Peluso, Lara Valeri, Sara Gavioli, Alberto Neri, Gabriele Trimarchi, Stefano Giuseppe Caraffi, Livia Garavelli, and Andrea Superti-Furga; molecular studies and bioinformatic analysis: Katarina Cisarova, Belinda Campos-Xavier, and Andrea Superti-Furga; writing—original draft preparation: Katarina Cisarova and Francesca Peluso; writing—review and editing: Andrea Superti-Furga, Livia Garavelli, and Giancarlo Gargano; supervision: Andrea Superti-Furga. All authors discussed, read and approved the manuscript in its final form.

DATA AVAILABILITY STATEMENT

The data supporting the findings in this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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