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Mandibulofacial Dysostosis with Microcephaly: Mutation and Database Update

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Abstract

Mandibulofacial dysostosis with microcephaly (MFDM) is a multiple malformation syndrome comprising microcephaly, craniofacial anomalies, hearing loss, dysmorphic features, and, in some cases, esophageal atresia. Haploinsufficiency of a spliceosomal GTPase, U5–116 kDa/EFTUD2, is responsible. Here, we review the molecular basis of MFDM in the 69 individuals described to date, and report mutations in 38 new individuals, bringing the total number of reported individuals to 107 individuals from 94 kindreds. Pathogenic *EFTUD2* variants comprise 76 distinct mutations and seven microdeletions. Among point mutations, missense substitutions are infrequent (14 out of 76; 18%) relative to stop-gain (29 out of 76; 38%), and splicing (33 out of 76; 43%) mutations. Where known, mutation origin was de novo in 48 out of 64 individuals (75%), dominantly inherited in 12 out of 64 (19%), and due to proven germline mosaicism in four out of 64 (6%). Highly penetrant clinical features include, microcephaly, first and second arch craniofacial malformations, and hearing loss; esophageal atresia is present in an estimated ~27%. Microcephaly is virtually universal in childhood, with some adults exhibiting late “catch-up” growth and normocephaly at maturity. Occasionally reported anomalies, include vestibular and ossicular malformations, reduced mouth opening, atrophy of cerebral white matter, structural brain malformations, and epibulbar dermoid. All reported *EFTUD2* mutations can be found in the *EFTUD2* mutation database (http://databases.lovd.nl/shared/genes/EFTUD2).

Keywords

EFTUD2; mandibulofacial dysostosis with microcephaly; MFDM; mandibulofacial dysostosis Guion-Almeida type; mandibulofacial dysostosis; microcephaly

Background

Mandibulofacial dysostosis with microcephaly (MFDM; MIM# 610536) is a multiple malformation syndrome comprising microcephaly, first and second branchial arch anomalies (Pierre-Robin sequence, malar hypoplasia, zygomatic clefting, microtia, middle ear

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Additional Supporting Information may be found in the online version of this article.

Ethical Compliance

Duly informed consent was obtained from all individuals participating in this study. The research protocol was approved by the Children’s Hospital of Eastern Ontario Research Ethics Board, and clinical data were obtained in a manner conforming with research ethics board and funding agency guidelines.

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malformations, choanal atresia, and/or auditory atresia), hearing loss, dysmorphic features, and variable systemic malformations (esophageal atresia, short stature, cardiac and/or genitourinary anomalies, and proximally-placed thumbs) [Guion-Almeida et al., 2006; Wieczorek et al., 2007; Wieczorek et al., 2009]. Haploinsufficiency of *EFTUD2* (MIM# 603892), encoding U5–116 kDa, a spliceosomal GTPase, is responsible [Lines et al., 2012]. The majority of described mutations are de novo, with a variety of documented mutation types (missense, nonsense, frameshift, splice site, and complete or partial gene deletions, including cytogenetically-visible deletions); however, autosomal dominant inheritance and, less commonly, germline mosaicism have been reported [Lines et al., 2014].

Variants

Previous to this report, the literature includes 69 individuals (64 kindreds) with mutations of *EFTUD2* (NM 004247.3), excluding individuals with cytogenetically visible chromosomal aberrations [Lines et al., 2012; Gordon et al., 2012; Need et al., 2012; Bernier et al., 2012; Luquetti et al., 2013; Voigt et al., 2013; Lehalle et al., 2014; Gandomi et al., 2015; Smigiel et al., 2015; Sarkar et al., 2015; Vincent et al., 2015]. Here, we summarize all published affected individuals and present genotypic and phenotypic data from a further 38 affected individuals belonging to 30 kindreds (Supp. Tables S1 and S2, Table 1, and Fig. 1). Across the entire series, the 83 distinct *EFTUD2* mutations are classified as follows: seven large (whole-gene or multi-exon) deletions, 16 frameshift, 12 nonsense, 32 splice site, one small deletion/duplication, 14 missense, and one intronic change previously suggested to create a novel splice donor site. Of 64 individuals for whom parental genotypes were available, de novo inheritance was demonstrated in 48 (75%), whereas mutations were inherited from an affected parent in 12 (19%), or via germline mosaicism in four (6%; two sibling pairs with confirmed paternity but normal parental sequencing) (Supp. Fig. S1). Three of the splice-site mutations presented here have further been validated by RT-PCR (Supp. Fig. S2).

Clinical Relevance

To better define the spectrum of *EFTUD2*-associated clinical symptoms, we reviewed the clinical features of all newly reported individuals in this study (Supp. Table S2), and tabulated the feature-specific penetrance of each of the major clinical findings across the entire cohort of all known affected individuals (Table 1) [Lines et al., 2012;Need et al., 2012; Bernier et al., 2012; Gordon et al., 2012; Voigt et al., 2013; Luquetti et al., 2013; Lehalle et al., 2014, Smigiel et al., 2015]. Highly penetrant features seen in >80% of individuals include developmental delay, microcephaly (here defined as occipitofrontal circumference (OFC) two or more standard deviations below mean), micrognathia, malar hypoplasia, small or dysplastic pinnae, and hearing loss (typically conductive, but occasionally sensorineural or mixed). Less frequent, but diagnostically useful, manifestations include preauricular tag(s) (52%), auditory canal atresia or stenosis (64%), tracheoesophageal fistula (TEF)/ esophageal atresia or stenosis (27%), thumb abnormalities (31%), and choanal atresia or stenosis (33%; likely under-reported). Zygomatic arch hypoplasia/clefting and middle ear abnormalities (hypoplastic, fused, or absent ossicles, complete absence of middle ear structures, or hypoplasia/absence of semicircular canals) appear to be relatively frequent, although these features, which are best appreciated by computed tomography, have not been

assessed systematically in most patients. The wide range of minor anomalies described in <10% of affected individuals includes: Renal anomalies (nineindividuals), cryptorchidism and/or small scrotum (seven), reduced mouth opening (seven), hemivertebrae or posterior dysraphism (six), cerebral and/or white matter atrophy (six), other CNS abnormalities (six; various including olfactory bulb agenesis; pontine hypoplasia; cerebellar hypoplasia, delayed gyration, delayed myelination, or exencephaly), absence of (naso-)lacrimal ducts (five), scoliosis/kyphosis (five), strabismus (four), absent 12th rib pair (four), epibulbar dermoid (three), branchial cleft and/or remnant (two), laryngeal cleft (one), midline mandibular defect (one), and gastric malrotation (one).

Although microcephaly was seen in all subjects in the original gene discovery cohort [Lines et al. 2012], several normocephalic individuals have since been reported [Gordon et al., 2012; Luquetti et al., 2013; Lehalle et al., 2014]. To study the penetrance of microcephaly in MFDM, we reviewed all available primary growth data in 57 affected individuals (Fig. 2). As described previously, microcephaly may be of either congenital or postnatal onset [Lines et al., 2012]. Despite the slower rate of cephalic growth during infancy and childhood, some affected individuals go on to exhibit gradual “catch-up” in OFC growth, with final adult OFC within the normal range. It may therefore be useful to review historical growth charts in normocephalic adults when considering the diagnosis of MFDM. Linear growth in MFDM may be normal or reduced (Fig. 2).

Although developmental delay is nearly uniform in MFDM, developmental outcomes vary significantly. The mean reported age of independent walking was 26.7 months in this cohort ($n = 23$), and 26.4 months for all patients (range: 13–60 months; $n = 38$). Among verbal individuals, the mean reported age of first meaningful word was 26.4 months ($n = 24$), and 27.4 months (range: 12 months to >5.5 years; $n = 32$) for all patients. Of adult patients in our cohort, one was a ward of the state, four lived with parents, one lived in a group home, and two lived in their own home, or with a spouse. At least one individual was able to carry out simple paid work outside of the home, and another was able to complete high school. Other individuals were nonverbal and dependent on caregivers for daily activities into adulthood, with significant behavioral difficulties. Of individuals for whom parental segregation analysis has been performed, dominant transmission was documented in 12 out of 64 meioses (19%), and germline mosaicism was proven in a further four out of 64 meioses (6%). These figures, which are higher than recognized previously, should be considered when counseling apparently unaffected parents with respect to recurrence risks.

Biological Relevance

In the *Saccharomyces cerevisiae* U4/U5/U6 small nuclear ribonucleoprotein complex (“tri-snRNP”), the EFTUD2 orthologue Snu114p bridges the U5-snRNP helicases Brr2p and Prp8p, and several subunits of the U4/U6 snRNP complex [Häcker et al., 2008]. By analogy with the ribosomal elongation factor EF-2, Snu114p has been proposed to undergo a significant GTP-dependent conformational change, allowing the dissociation of U5 and U4/U6 snRNPs during splicing. Protein-protein and protein–GTP interactions are therefore both essential to EFTUD2’s function. In a structural model of EFTUD2, four [p.(His208Arg), p.(Lys620Asn), p.(His856Tyr), and p.(Arg938His)] of 14 described missense

substitutions in MFDM alter basic, surface-forming residues that are potentially available for protein–protein interactions (Fig. 3). A fifth mutation, p.(Arg262Trp), is a recurrent mutation of a conserved residue in EFTUD2's GTP-binding site observed in three unrelated families [Lines et al., 2012; Smigiel et al., 2015; this study]. The remaining nine missense substitutions, which are predicted to be interior to EFTUD2, could conceivably affect protein stability by any of several mechanisms, including effects on: protein stability, conformation, localization, and/or post-translational modifications.

Considering the entire allelic series for *EFTUD2*, the high proportion of truncating (frameshift, nonsense, or splice-site) mutations, and several instances of whole-gene or multi-exon deletions, suggests haploinsufficiency to be the predominant mechanism. How partial deficiency of a ubiquitously expressed spliceosomal subunit causes the specific pattern of malformations seen in MFDM is unknown. Craniofacial anomalies similar to those seen in MFDM are observed in other disorders of ribonucleoprotein metabolism and/or ribosome biogenesis, including: Nager syndrome (*SF3B4*) [Bernier et al., 2012], Cerebro-Costo-Mandibular syndrome (*SNRPB*) [Lynch et al., 2014], Richieri-Costa-Pereira syndrome (*EIF4A3*) [Favaro et al., 2014], Treacher Collins syndrome (*TCOF1, POLR1C, POLR1D*) [Treacher Collins Syndrome Collaborative Group 1996; Dauwerse et al., 2011], and Diamond-Blackfan anemia (multiple ribosomal genes) [Ruggero and Shimamura, 2014; Gripp et al., 2014]. In Treacher Collins syndrome, the prototype for this group of disorders, "nucleolar stress" as a result of impaired ribosome biogenesis causes p53-dependent attrition of a specific population of neural crest cells destined for the first and second arches [Dixon et al., 2006; Jones et al., 2008]. Similarly, in a *Danio rerio* morpholino model of Richieri-Costa-Pereira syndrome, deficiency of Eif4a3, an exon-junction protein deposited after splicing events, produces similar effects on the developing cranial neural crest [Singh et al., 2012; Favaro et al., 2014]. Craniofacial defects are therefore common to disorders of several different types of ribonucleoprotein complexes (ribosome, spliceosome, and exon–junction complex), and the nucleotide precursor defects Miller Syndrome (*DHODH*) and methotrexate toxicity [Ng et al., 2010], although the intermediate steps in this pathway are obscure at present.

Database

In order to establish a central repository for all known *EFTUD2* mutations, we (D.B.) maintain the *EFTUD2* Mutation Database (http://databases.lovd.nl/shared/genes/EFTUD2), according to the Leiden Open Variation Database (LOVD) system(currently version 3.0, build 12) [Fokkema et al., 2011]. As far as we are aware, all described mutations have been deposited in the *EFTUD2* Mutation Database.

Diagnostic Relevance

The experience with *EFTUD2* testing in MFDM suggests that sensitivity of sequencing and deletion testing approaches 100%.We are aware of only one described case of apparent nonpenetrance: the paternally-inherited deep-intronic change (c.2347+66A>G) reported in Gordon et al. (2012), for which he have neither a detailed clinical description of the parent in question, nor confirmatory RT-PCR studies to verify abnormal splicing. We consider

individual #12 from the same publication to have another diagnosis due to the lack of characteristic features or a pathogenic *EFTUD2* variant (see legend of Supp. Table S1 for details). Therefore, to our knowledge, there are no proven instances of nonpenetrance in an individual with a bona fide *EFTUD2* mutation. The presence of an *EFTUD2* mutation compatible with haploinsufficiency, particularly when de novo, is therefore highly compelling evidence to establish the diagnosis, although this can equally be determined based on clinical criteria [Lines et al., 2014].

Future Prospects

Because of the small number of total cases reported to date, and inherent clinical bias towards ascertaining "classic" (i.e., severe) cases, it is tempting to speculate that milder or attenuated *EFTUD2* phenotypes, such as those with comparatively mild missense substitutions, may remain to be identified. The inclusion of *EFTUD2* on next-generation-based sequencing panels should facilitate the recognition of the full clinical spectrum of MFDM. The main biological questions to be investigated include (i) which RNA transcripts are relevant to the developmental abnormalities seen in MFDM patients, and (ii) whether/how abnormal splicing produces "nucleolar stress" (impaired ribosome biogenesis) in the developing first- and second-arch-destined neural crest. Because this is a disorder characterized by haploinsufficiency, the phenotypic effect of pharmacologically or otherwise increasing the expression of the remaining *EFTUD2* allele in a relevant animal model may warrant investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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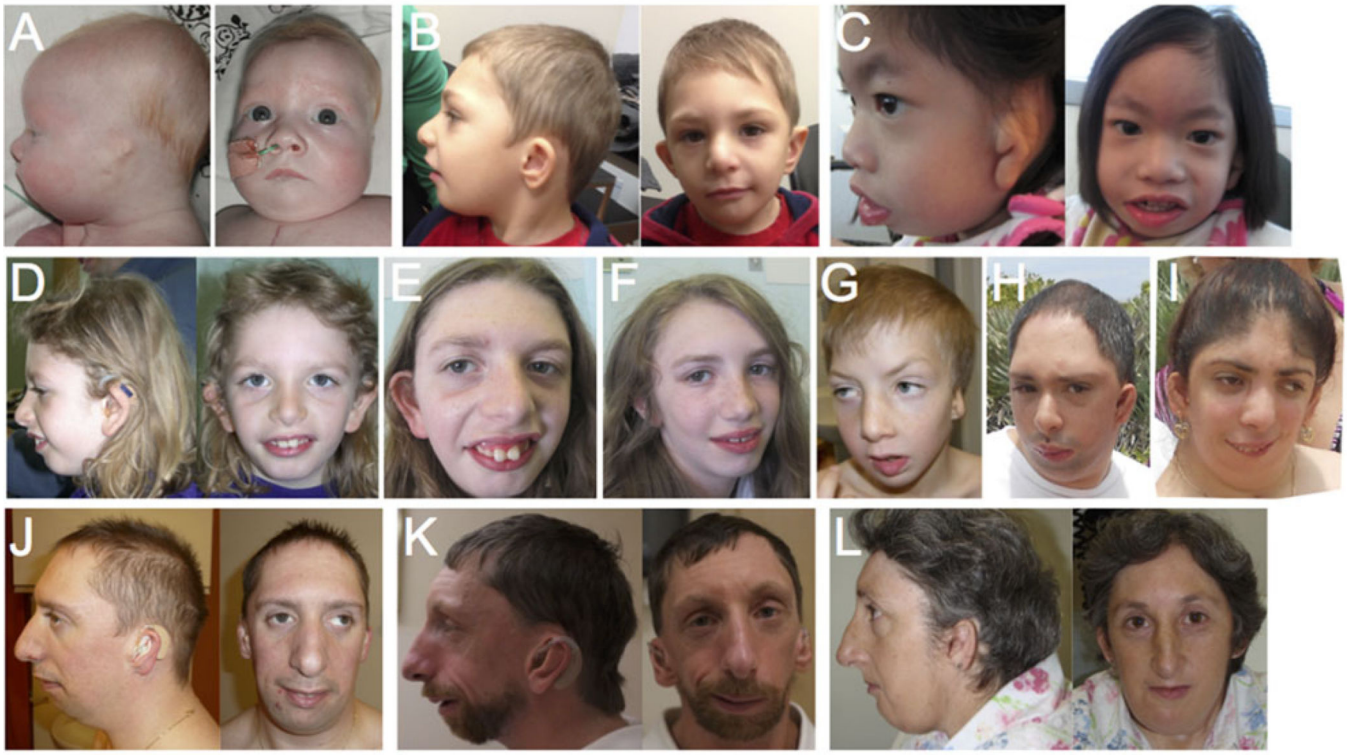

Figure 1.

Craniofacial morphology in children and adults with mutations in *EFTUD2*. Panel **A**: Individual #62 (age 4 months); **B**: #52 (3.25 years); **C**: #60 (3.5 years); **D**: #10a (age 4.5 years); **E**: #10a (15 years); **F**: #10b (12 years); **G**: #57 (age not specified); **H**: #79a (28 years); **I**: #79b (25 years); **J**: #12 (31 years); **K**: #13 (43 years); **L**: #34 (47 years). The typical gestalt associated with MFDM (convex facial profile with micrognathia, midface hypoplasia, and sloping forehead, strong supraorbital ridges, high nasal bridge with a prominent ridge and rounded tip, ear anomalies including microtia, dysplastic pinnae, deficient superior helix, posteriorly “squared” earlobes, and/or preauricular tags), while seen in the majority of affected individuals, may occasionally be subtle (e.g., panels **F** and **L**).

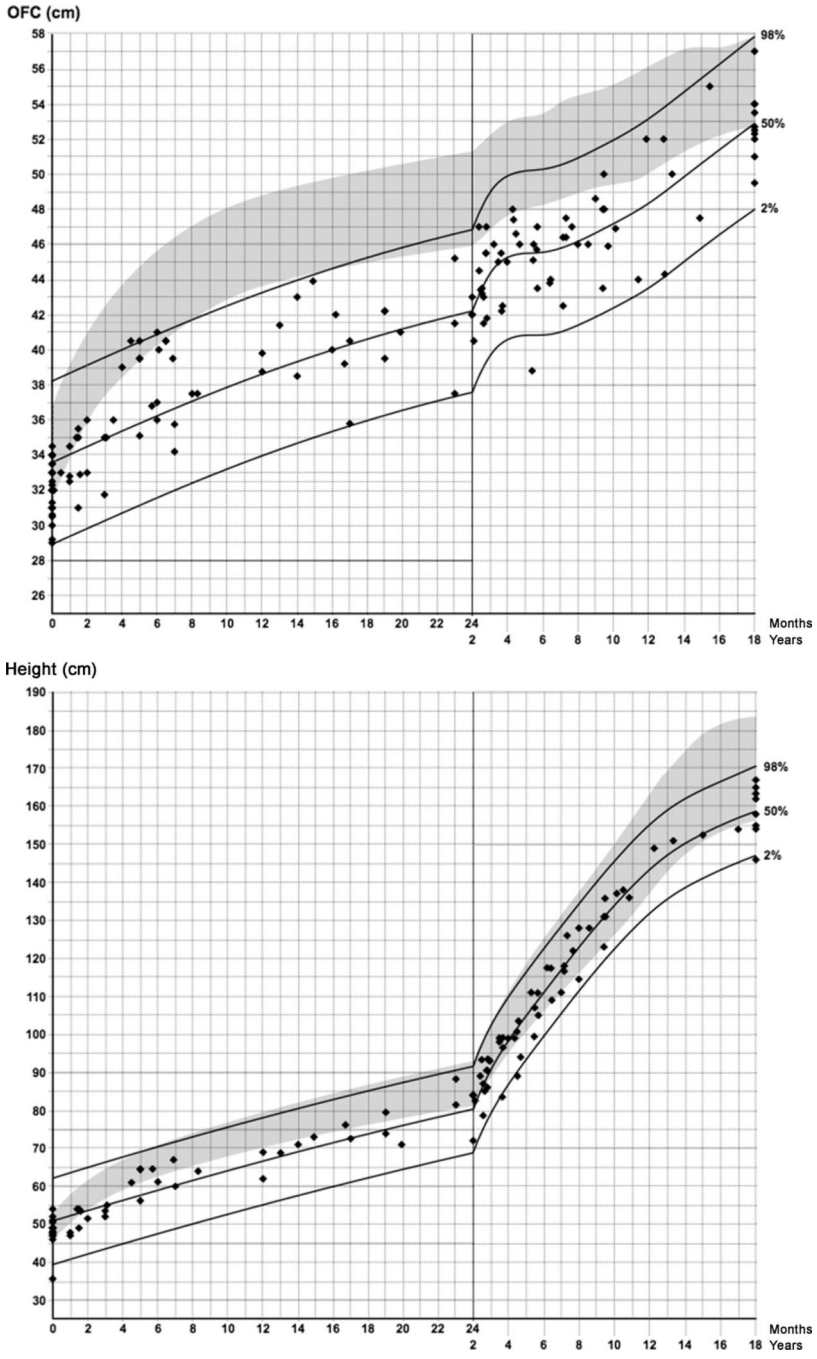

Figure 2.

Growth in MFDM. Primary growth data from 34 previously unpublished affected individuals, and 23 published affected individuals [Lines et al., 2012, Luquetti et al., 2013, Voigt et al., 2013, Sarkar et al., 2015] were modelled using the LMS method, based on a local generalized AIC criterion with smoothing penalty $k = 10$. The 2nd, 50th, and 98th percentile LMS curves (solid lines) are as shown. Each point represents an individual measurement from an affected individual; dates were corrected for prematurity if applicable. Top: Occipitofrontal circumference (OFC). Shaded area represents sex-averaged general

population reference (−2SD to +2SD) [Nellhaus G, 1968]. Bottom: Linear growth versus general sex-averaged general population reference (3rd to 97th centile) from 2014 World Health Organization Growth Charts for Canada (http://www.whogrowthcharts.ca).

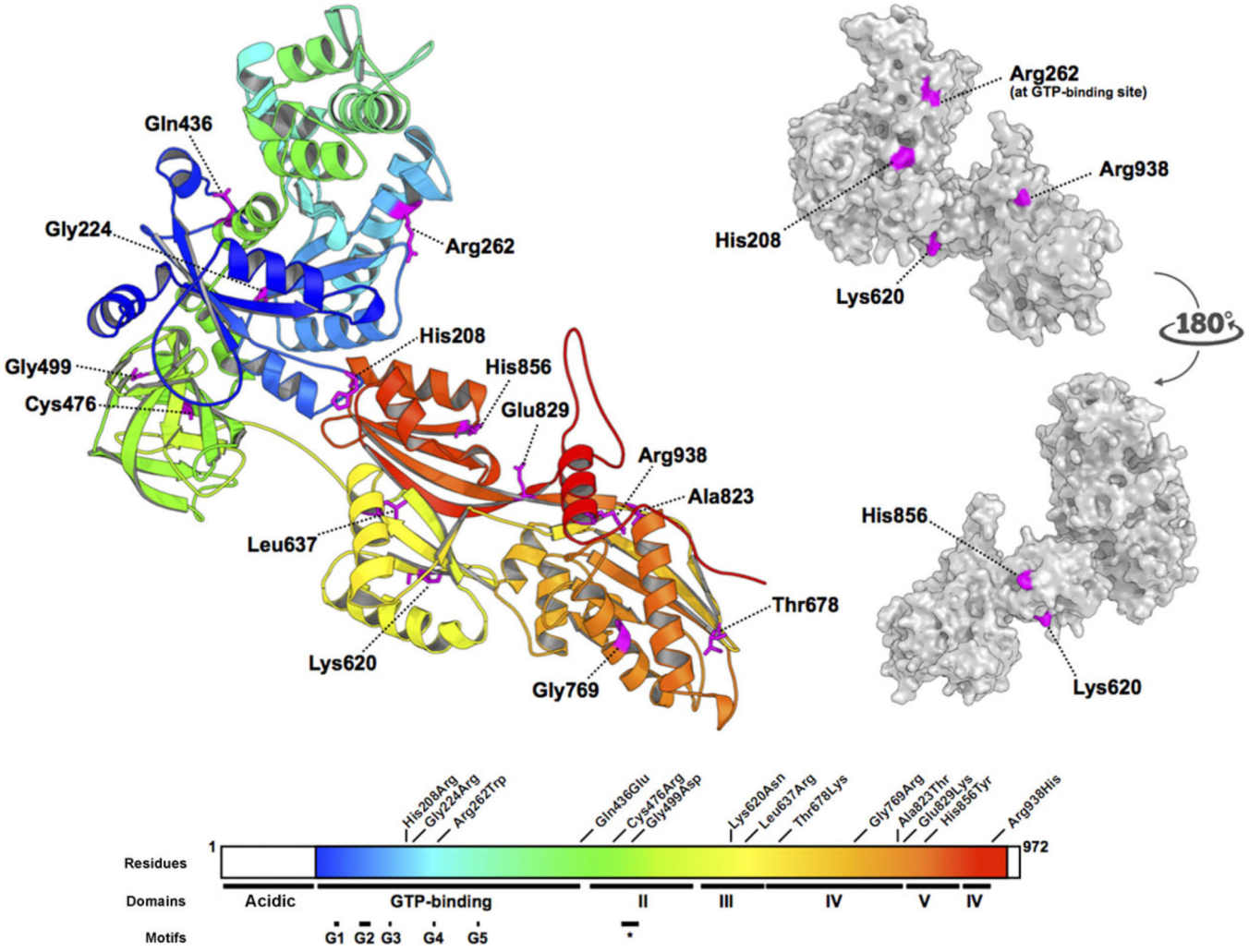

Figure 3.

All *EFTUD2* missense substitutions described to date. (Based on Fabrizio et al., 1997 and Lines et al., 2012). Residues 114–957 of EFTUD2 were modelled on the crystal structure of *S. cerevisiae* ribosomal elongation factor 2 (eEF2) (PDB: 1N0U). Missense substitutions are distributed throughout all domains of the protein. Conserved motifs identified by Fabrizio et al. (1997), including the GTP-binding domains G1 through G5, and the short conserved domain II motif (∗), are shown. The side chains of His208, Arg262, Lys620, and His856, and Arg938, all basic residues, are predicted to be surface-forming, whereas the sidechains of Gln436, Leu637, Thr678, and Glu829, Gly224, Cys476, Gly499, Gly769, and Ala823 are interior to the model.

Table 1

Penetrance of Clinical Findings in MFDM

Feature	This study	All reported individuals[a]	Estimated penetrance (%)	95% CI (%)
Craniofacial				
Micrognathia	34/34	87/89	98	92–99
Small or dysplastic pinna(e)	31/34	84/87	97	90–99
Malar hypoplasia	34/36	78/84	93	85–97
Hearing loss	31/35	69/83	83	74–90
Conductive	16/27	32/51	63	49–75
Mixed	10/27	13/51	25	16–39
Sensorineural	1/27	6/51	12	6–23
Auditory atresia/stenosis	19/29	47/73	64	53–74
Vestibular system abnormalities	1/8[b]	14/25[b]	56[b]	37–73[b]
Ossicular abnormalities	4/8[b]	8/15[b]	53[b]	30–75[b]
Facial asymmetry	nr	25/47	53	39–67
Preauricular tag(s)	19/34	45/86	52	42–63
Cleft palate	20/35	41/88	47	37–57
Choanal atresia	6/34	27/83	33	23–43
Neonatal resuscitation	9/32	14/46	30	19–45
Tracheostomy	7/34	10/50	20	11–33
Extracranial				
Thumb anomalies	10/32	24/77	31	22–42
Heart defects	9/38	28/89	31	23–42
Esophageal atresia/TEF	5/35	23/85	27	19–37
Development				
Developmental delay	31/31	83/83	100	96–100
Microcephaly	27/33	78/89	88	79–93
Congenital	10/18	34/53	64	51–76
Postnatal	8/18	19/53	36	24–49
Seizures	9/30	21/77	27	19–38

[a] Includes cases in: Lines et al. (2012), Need et al. (2012), Bernier et al. (2012), Gordon et al. (2012) (excluding individual #12), Voigt et al. (2013), Luquetti et al. (2013), Lehalle et al. (2014), Smigiel et al. (2015), and this study.

[b] This feature has not been assessed in the majority of reported patients (estimate is likely inaccurate due to ascertainment bias).