Review

A review of craniofacial disorders caused by spliceosomal defects


The spliceosome is a large ribonucleoprotein complex that removes introns from pre-mRNA transcripts. Mutations in EFTUD2, encoding a component of the major spliceosome, have recently been identified as the cause of mandibulofacial dysostosis, Guion-Almeida type (MFDGA), characterized by mandibulofacial dysostosis, microcephaly, external ear malformations and intellectual disability. Mutations in several other genes involved in spliceosomal function or linked aspects of mRNA processing have also recently been identified in human disorders with specific craniofacial malformations: SF3B4 in Nager syndrome, an acrofacial dysostosis (AFD); SNR PB in cerebrocostomandibular syndrome, characterized by Robin sequence and rib defects; EIF4A3 in the AFD Richieri-Costa–Pereira syndrome, characterized by Robin sequence, median mandibular cleft and limb defects; and TXNL4A in Burn-McKeown syndrome, involving specific craniofacial dysmorphisms. Here, we review phenotypic and molecular aspects of these syndromes. Given the apparent sensitivity of craniofacial development to defects in mRNA processing, it is possible that mutations in other proteins involved in spliceosomal function will emerge in the future as causative for related human disorders.

Conflict of interest

The authors have no conflict of interest to declare.

Mandibulofacial dysostosis (MFD) is a malformative craniofacial disorder characterized by malar and mandibular hypoplasia as the core phenotype, with dysplastic ears and eyelids, hearing loss and cleft palate as frequently associated features (1). MFD is a part of several genetically defined syndromes, each with a distinctive combination of features. One of the most frequent MFDs is Treacher Collins syndrome (TCS; MIM 154500), in which defects are typically restricted to the craniofacial complex. Most TCS patients have heterozygous, de novo, loss-of-function mutations in TCOF1, which encodes Treacle, a protein involved in rDNA transcription and hence ribosome biogenesis (2). Heterozygous, homozygous POLR1D or compound heterozygous POLRIC mutations have also been identified in TCS patients (3, 4). Forms of MFD associated with limb anomalies are known as acrofacial dysostoses (AFDs) (1). Nager syndrome (NS; MIM 154400) is an AFD that includes preaxial upper limb defects and a craniofacial phenotype that overlaps TCS (Fig. 1). NS is caused by mutations in SF3B4, encoding a spliceosomal protein (5). Miller syndrome (MIM 263750), an AFD characterized by postaxial limb defects and MFD, is caused by biallelic mutations in the gene coding for...
dihydroorotate dehydrogenase (DHODH), resulting in a deficiency in de novo pyrimidine biosynthesis (6, 7). Miller syndrome and TCS are not spliceosomopathies (the focus of this review) and are therefore not discussed further here. A more recently delineated, although relatively frequent syndrome is mandibulofacial dysostosis, Guion-Almeida type (MFDGA; MIM 610536), which is characterized by MFD, external ear malformations, microcephaly and intellectual disability (Fig. 1). MFDGA is caused by heterozygous loss-of-function mutations or deletions of EFTUD2, encoding a component of the spliceosome (8).

The genes identified in the MFD syndromes described above are expected to take part in widespread cellular events; the phenotypic restriction is therefore surprising. Along the same lines, defects in mRNA processing have recently been established as the basis of two syndromes in which the mandible is severely affected. Cerebrocostomandibular syndrome (CCMS; MIM 117650)-affected individuals exhibit Robin sequence and characteristic rib defects and harbor mutations in SNRPB (9, 10), while Richieri-Costa–Pereira syndrome (RCPs; MIM 268305) is characterized by midline mandibular cleft, Robin sequence, radial and tibial abnormalities, and often intellectual disability, and is associated with mutations in EIF4A3 (11) (Fig. 1). Finally, mutations in TXNL4A, encoding a protein of the major spliceosome, have been identified as the cause of Burn-McKeown syndrome (BMKS; MIM 608572), with choanal atresia, hearing loss, lowered eyelid defects and cleft lip or palate as major features (12) (Fig. 1). Most of the above disorders have been genetically defined in the last 3 years by exome/genome sequencing. In this review we focus on five disorders with major craniofacial involvement: MFDGA, NS, CCMS, RCPs and BMKS – all caused by mutations in factors that play a role within, or are functionally linked to, the spliceosome.

The spliceosome in human disease

The splicing of introns from pre-mRNAs is a fundamental prerequisite for translation of mRNA into protein in eukaryotic cells, and is performed by the spliceosome, a ribonucleoprotein complex containing more than 170 proteins (13, 14). Splicing is achieved in multiple steps, commencing with recruitment of the spliceosomal machinery to cis-acting motifs within the pre-mRNA: the 5′ and 3′ splice sites at either end of an intron and the branch site, which is a short distance upstream of the 3′ splice site. The majority of introns are spliced by the major spliceosome, which consists of several key RNA-protein complexes, the U1, U2, U4/U6 and U5 small nuclear ribonucleic proteins (snRNPs; Fig. 2), while the U12-dependent minor spliceosome is comprised of U11, U12, U5, U4atac and U6atac snRNPs and splices only about 700–800 introns. Initial steps of splicing by the major spliceosome involve the interaction of the U1 snRNP with the 5′ splice site and of the U2 snRNP with the branch site. Subsequently, the preassembled U4/U6-U5 tri-snRNP is recruited, and U1 and U4 are then displaced. The resulting arrangement, with the U6 and U2 snRNAs basepaired to each other and to the 5′ and 3′ splice sites, respectively, and the U5 snRNA basepaired to exonic sequences upstream of the 5′ splice site, is the catalytically active form of the spliceosome. Catalysis then proceeds in two steps: first, the 5′ end of the intron is cleaved and joined to the branch site to create a lariat structure and secondly the 3′ end of the intron is cleaved, allowing ligation of the 5′ and 3′ exons (13, 14).

Somatic mutations of the spliceosomal machinery have been described in cancers such as myelodysplastic syndrome and chronic lymphocytic leukemia (15), while germline mutations in genes encoding proteins or RNAs of the spliceosome have been identified in conditions with autosomal recessive or autosomal dominant modes of inheritance. Of the many genes associated with retinitis pigmentosa (MIM 268000), which involves retinal degeneration due to progressive photoreceptor cell loss, autosomal dominant mutations have been found in PRPF3, PRPF4, PRPF6, PRPF8, PRPF31, RP9 and SNRNPR200 (16–22). These genes encode factors involved in assembly or function of the U4/U6-U5 tri-snRNP of the spliceosome (23). In the genodermatosis poikiloderma with neutropenia, Clericiuzio-type (MIM 604173), mutations have been identified in USB1 (24), encoding an enzyme required for processing of the U6 snRNA (25, 26). A subset of amyotrophic lateral sclerosis patients have mutations in TARDBP or FUS (MIM 612069, MIM 608030) and SMN1 mutations cause spinal muscular atrophy (MIM 253300). These motor neuron diseases may have defects in snRNP biogenesis as a common underlying mechanism (27). It has recently been shown that the product of RBM10, which is mutated in TARP syndrome (MIM 311900, consisting of talipes equinovarus, atrial septal defect, Robin sequence and persistence of left superior vena cava) (28), associates with spliceosomal complexes, binds RNAs in the vicinity of splice sites and regulates alternative splicing by enhancing exon skipping (29). Mutations in RN44ATAC, an RNA component of the minor spliceosome, cause Taybi-Linder syndrome (TALS; MIM 210710), also called microcephalic osteodysplastic primordial dwarfism type 1 (30, 31). TALS is a severe developmental disorder in which the major features are pre- and post-natal growth retardation, microcephaly, bone dysplasia and brain malformations. It is probable that other syndromes with a variable spectrum of congenital malformations will be ascribed to dysfunction of the spliceosome in the future.

Interestingly, despite the fact that RNA splicing is a ubiquitous and essential process, several of the above conditions affect one or a limited number of tissues. The mechanisms underlying pleiotropic versus tissue-specific effects caused by disruption of spliceosomal function are not yet fully understood. One hypothesis is non-canonical and unknown functions for some components of the spliceosome. Differing rates of transcript and translation among different cell types may also play a role. Along these lines, it has been suggested that the retina has a higher dependence on spliceosomal function than other tissues, leading to the phenotypic restriction seen in retinitis pigmentosa,
Craniofacial disorders and the spliceosome

Fig. 1. Facial features of individuals presenting with the disorders described in this review. (a, b) Mandibulofacial dysostosis, Guion-Almeida type (MFDGA) [previously reported as case 31 in reference (40)]. (c, d) MFDGA [previously reported as case 17 in reference (40)]. (e, f) Nager syndrome (NS) (reproduced from the Robert J. Gorlin slide collection). (g–i) Cerebrocostomandibular syndrome [previously reported as patient 1 in reference (10)]. (j, k) RCPS [previously reported as patient 22 in reference (11)]. (l, m) RCPS [previously reported as patient 15 in reference (11)]. (n, o) BMKS [previously reported as patient II1 of family BMKS004 in reference (12)].
Fig. 2. Core components of the human major spliceosomal snRNPs, and of the exon junction complex (EJC). The factors involved in the disorders reviewed here are circled in black (craniofacial disorders, solid lines; other disorders, dashed lines). MFDGA, mandibulofacial dysostosis, Guion-Almeida type; NS, Nager syndrome; CCMS, cerebrocostomandibular syndrome; BMKS, Burn-McKeown syndrome; RCPS, Richieri-Costa–Pereira syndrome; IH, isolated hypotrichosis; TAR, thrombocytopenia with absent radius syndrome; RP, retinitis pigmentosa. Figure adapted from reference (13). Nomenclature of the HUGO Gene Nomenclature Committee is used. The seven Sm proteins are common to all snRNPs.

even though the splicing defects may be ubiquitous (32). However if this was the sole explanation, all spliceosomalopathies should display retinal degeneration as a component of the phenotype. In a mouse model of SMA, Smn mutant mice have widespread defects in splicing, but surprisingly have tissue-specific alterations in the repertoire of affected snRNAs and mRNAs, potentially accounting for the motor neuron-specific pathology of SMA (33). The dosage of spliceosomal components may also influence the tissue-specificity of a phenotype. Indeed, patients with congenital SMA who are null for SMN1 and have only one copy of the SMN1 parologue SMN2 present more often with congenital heart defects in addition to the neurological phenotype than SMN1-null patients with two or three copies of SMN2 (34). Studies in yeast and Drosophila have shown that loss of core spliceosomal factors can differentially perturb splicing of specific mRNAs (35, 36). Indeed, the modulation of wildtype spliceosomal activity across cell types and developmental stages may be an important mechanism for the regulation of gene expression.

**Mandibulofacial dysostosis Guion-Almeida type**

In 2012, mutations in EFTUD2 were identified via exome sequencing as the cause of MFDGA (8), and to date, more than 50 probands with EFTUD2 loss-of-function mutations or deletions have been described (5, 8, 37–44). Major features of MFDGA are MFD, external ear malformations, microcephaly and intellectual disability, while less frequent features include hearing loss, cleft palate, choanal atresia, oesophageal atresia, congenital heart defects and radial ray defects (Table 1). Alternative names for this syndrome are MFD with microcephaly or AFD type Guion-Almeida (the latter given the presence of limb anomalies (1)).

Although MFDGA now appears to be one of the most frequent MFDs, it was only recently delineated and the identification of the disease-causing gene has led to an expansion of the spectrum of congenital malformations ascribed to EFTUD2 loss-of-function. Prior to gene identification, MFDGA was recognized as a distinct syndrome in several patients reported by Guion-Almeida et al. and Wieczorek et al. in 2006 and 2009 (45–47). EFTUD2 turned out to be the causative gene for two other apparently unique syndromes (37): the syndrome described by Wieczorek et al. as ‘Esophageal Atresia, Hypoplasia of Zygomatic Complex, Microcephaly, Cup-Shaped Ears, Congenital Heart Defect, and Mental Retardation’ (48), and the oto-facial syndrome described by Megarbane et al. (49). In retrospect, the affected individuals of both families display typical features of MFDGA. In the latter family, although the syndrome affected two sibs born to consanguineous parents and was hence suggestive of an autosomal recessive mode of inheritance, a de novo heterozygous mutation in each of the sibs indicated that this was the first example of germinal mosaicism for an EFTUD2 mutation (37). Patients with differential diagnoses of Treacher Collins, Nager, CHARGE or Feingold syndromes or oculoauriculovertebral spectrum have been found to have EFTUD2 mutations (5, 38, 43, 47). One illustration of the heterogeneity in MFDGA came from an exome-sequencing study of 12 trios, the
## Table 1. Summary of clinical features of MFDGA, NS, CCMS, RCPS and BMKS

<table>
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<th>Syndrome</th>
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<td><strong>Craniofacial phenotype</strong></td>
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<tr>
<td>Mandibular hypoplasia</td>
<td>58/62 (94%)</td>
<td>8, 37-49</td>
<td>21/21 (100%)</td>
<td>6, 61, 62</td>
<td>16/18 (89%)</td>
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<td>25/25 (100%)</td>
<td>11</td>
<td>9/14 (64%)</td>
<td>12</td>
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<td>Malar hypoplasia</td>
<td>46/50 (92%)</td>
<td>5</td>
<td>17/19 (89%)</td>
<td>6, 61, 62</td>
<td>2/13 (15%)</td>
<td>5</td>
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<td>External ear anomalies</td>
<td>61/63 (87%)</td>
<td>61, 62</td>
<td>15/18 (83%)</td>
<td>6, 61, 62</td>
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<td>5</td>
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<td>Preauricular tags</td>
<td>31/35 (65%)</td>
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<td>4/11 (49%)</td>
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<tr>
<td>Hearing loss</td>
<td>43/65 (78%)</td>
<td>27, 61</td>
<td>1/7</td>
<td>27</td>
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<td>2</td>
<td>6/18 (30%)</td>
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<td>10/14 (71%)</td>
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<td>Auditory canal stenosis</td>
<td>30/51 (61%)</td>
<td>27, 61</td>
<td>4/4 (49%)</td>
<td>27</td>
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<td>2</td>
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<td>2</td>
<td>10/14 (71%)</td>
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<tr>
<td>Choanal atresia</td>
<td>22/62 (35%)</td>
<td>27, 61</td>
<td>–</td>
<td>27</td>
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<td>2</td>
<td>10/14 (71%)</td>
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<tr>
<td>Cleft palate/bifid uvula</td>
<td>22/61 (36%)</td>
<td>27, 61</td>
<td>8/13 (62%)</td>
<td>27</td>
<td>14/18 (78%)</td>
<td>2</td>
<td>18/21 (86%)</td>
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<td>7/14 (50%)</td>
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<tr>
<td>Facial asymmetry</td>
<td>27/49 (55%)</td>
<td>27, 61</td>
<td>2/7 (29%)</td>
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<td>2</td>
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<td>2</td>
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<tr>
<td>Eye anomalies</td>
<td>10/33 (30%)</td>
<td>27, 61</td>
<td>–</td>
<td>27</td>
<td>1/13</td>
<td>2</td>
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<tr>
<td>Lower eyelid defects</td>
<td>–</td>
<td>27, 61</td>
<td>11/16 (69%)</td>
<td>27</td>
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<tr>
<td>Semicircular canal an.</td>
<td>11/15 (73%)</td>
<td>27, 61</td>
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<td>27</td>
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<td>2</td>
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<tr>
<td>Cleft lip</td>
<td>–</td>
<td>27, 61</td>
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<td>27</td>
<td>1/13</td>
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<tr>
<td>Microstomia</td>
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<td>27</td>
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<td>Abnormal larynx</td>
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<td>27</td>
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<td>Absent lacrymal duct</td>
<td>1/59</td>
<td>27, 61</td>
<td>1/7</td>
<td>27</td>
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<tr>
<td>Temporo-mandibular an.</td>
<td>–</td>
<td>27, 61</td>
<td>5/5 (100%)</td>
<td>27</td>
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<td>2</td>
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<tr>
<td>Microcephaly</td>
<td>57/64 (89%)</td>
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<td>27</td>
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<td>Psychomotor delay/ID</td>
<td>59/60 (98%)</td>
<td>27, 61</td>
<td>2/14 (14%)</td>
<td>27</td>
<td>3/17 (18%)</td>
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<td>50%</td>
<td>2</td>
<td>3/14 (21%)</td>
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<td>Brain MRI anomalies</td>
<td>9/19 (47%)</td>
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<tr>
<td>Epilepsy</td>
<td>13/66 (23%)</td>
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<td>27</td>
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<tr>
<td>Autism</td>
<td>1/55</td>
<td>27, 61</td>
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<td>27</td>
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<td>Upper limb anomalies</td>
<td>27/62 (44%; thumbs 34%)</td>
<td>27, 61</td>
<td>21/21 (thumbs 100%)</td>
<td>2</td>
<td>25/25 (100% radial ray)</td>
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<td>25/25 (tibia 100%, club foot 96%)</td>
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<tr>
<td>Lower limb anomalies</td>
<td>6/62 (10%) (minor feet anomalies)</td>
<td>27, 61</td>
<td>8/21 (feet 100%)</td>
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<tr>
<td>Radio-ulnar synostosis</td>
<td>1/62</td>
<td>27, 61</td>
<td>7/20 (35%)</td>
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<td>Growth retardation</td>
<td>13/62 (21%)</td>
<td>27, 61</td>
<td>–</td>
<td>27</td>
<td>6/14 (43%)</td>
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<td>3/14 (21%)</td>
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<td>Congenital heart defect</td>
<td>23/58 (40%) (VSD, ASD, PDA)</td>
<td>27, 61</td>
<td>5/20 (25%) (AVSD, PDA, T4F)</td>
<td>2</td>
<td>3/13 (23%) (ASD)</td>
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<td>4/14 (29%) (PDA, PFO)</td>
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<td>Visceral malformations</td>
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<td>Oesophageal atresia</td>
<td>23/63 (37%)</td>
<td>27, 61</td>
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<td>27</td>
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<td>Anus stenosis</td>
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<td>27, 61</td>
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<td>27</td>
<td>1/13</td>
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<td>Rib gaps</td>
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<td>27, 61</td>
<td>–</td>
<td>27</td>
<td>13/18 (72%)</td>
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<td>Bell-shaped thorax</td>
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<td>27, 61</td>
<td>–</td>
<td>27</td>
<td>6/12 (50%)</td>
<td>2</td>
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<tr>
<td>Scoliosis</td>
<td>2/56 (4%)</td>
<td>27, 61</td>
<td>7/18 (39%)</td>
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<tr>
<td>Genito-urinary defects</td>
<td>11/61 (18%)</td>
<td>27, 61</td>
<td>1/3 (renal agenesis)</td>
<td>2</td>
<td>–</td>
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<td>1/14 (renal agenesis)</td>
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An., anomaly; ASD, atrio-septal defect; AVSD, atrio-ventriculo-septal defect; BMKS, Burn-McKeown syndrome; CCMS, cerebrocostomandibular syndrome; ID, intellectual disability; MFDGA, mandibulofacial dysostosis Guion-Almeida type, NS, Nager syndrome, PDA, persistent ductus arteriosus, PFO, persistent foramen ovale, RCPS, Richieri-Costa–Pereira syndrome; T4F, tetralogy of Fallot; VSD, ventriculo-septal defect.

The most frequent features of each syndrome, observed in more than 85% of affected individuals, are highlighted in bold.
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probands of each trio being a sporadic multiple congenital anomalies and/or mental retardation patient with no clinical diagnosis (39). Need et al. found de novo heterozygous variants in EFTUD2 in two patients, although they had not initially been considered similar clinically. The newly described entity Diamond-Blackfan Anemia with Mandibulofacial Dystostosis (DBA-MFD; MIM 606164) overlaps MFDGA, with malar and mandibular hypoplasia, ear anomalies, hearing loss, cleft palate and microcephaly reported in both conditions (50). Of note, hematological symptoms begin very early in DBA-MFD, with a severe anemia usually noticed in the first weeks or months of life. DBA-MFD is caused by mutations in multiple ribosomal factors (50), highlighting the potential for similar craniofacial phenotypes between ribosomopathies (TCS and DBA-MFD) and splicingopathies. As the number of published MFDGA patients has increased, a distinct facial gestalt with typically formed earlobes has become evident (40), enabling more accurate prediction of whether EFTUD2 is the likely cause. However, we and others have identified several patients with a phenotype strongly suggestive of MFDGA but without detection of an EFTUD2 point mutation. Genetic heterogeneity is therefore possible, although our initial analysis indicates that MLPA is effective for detection of intragenic CNVs in EFTUD2 in some point mutation-negative MFDGA patients (J.A., unpublished data).

EFTUD2 encodes a spliceosomal GTPase that is highly conserved across eukaryotes. Studies of Snu114p, the Saccharomyces cerevisiae ortholog of EFTUD2, have revealed that it plays important roles during several steps of the spliceosome cycle (51). Mutagenesis of Snu114p causes lethality or temperature-sensitive growth defects in yeast, with consequent defects in RNA splicing (52–56). Snu114p/EFTUD2 is a core component of the U5 snRNP, and interacts both physically and genetically with the RNA helicases Brr2p and Prp8p (the yeast orthologs of the retinopathy genes SNRNP200 and PRPF8, respectively) (55, 57–59). Prior to catalysis, Snu114p is involved in U4/U6 snRNA dissociation (53), and Snu114p also regulates disassembly of spliceosome subunits post-catalysis (60). Considering the close relationship of Snu114p with Brr2p and Prp8p in the tri-snRNP, the phenotypic discrepancy between MFDGA and retinitis pigmentosa is all the more remarkable. It will be of interest to test for retinal degeneration in MFDGA patients in later life.

Nager syndrome

NS is a rare AFD characterized by the association of MFD and limb defects, typically involving the radial ray (Table 1). The facial phenotype overlaps the gestalt of TCS, and is comprised of malar and mandibular hypoplasia, down-slanting palpebral fissures, lower eyelid and eyelash anomalies, external ear dysplasia and cleft palate (5, 61, 62). The most frequent limb abnormalities involve the thumbs, which can be hypoplastic, absent, or triphalangeal; less commonly seen are radioulnar synostosis, phocomelia, or lower limb defects (5, 61, 62). Psychomotor development can be mildly delayed; however the occipito-frontal circumference is usually within normal ranges. Other visceral malformations are rare. Although most instances are sporadic or consistent with autosomal dominant inheritance, a few examples suggestive of autosomal recessive inheritance have been reported (63, 64). In 2012, exome sequencing led to the identification of mutations in SF3B4 in 20 of 35 NS families (5). Almost all mutations identified were frameshift or nonsense, and a Nager-like individual with a proximal deletion of chromosome 1q, possibly encompassing SF3B4, had previously been reported (65), suggesting haploinsufficiency of SF3B4 as the cause (5). Since then, SF3B4 has been confirmed as the major disease-causing gene in dominant forms of NS, with mutations found in 18 more families (61, 62, 66, 67). Notably, more than a third of tested NS patients do not have SF3B4 mutations, suggesting that other loci for this disorder remain to be identified (5, 61, 62). Interestingly, one patient with a SF3B4 mutation, presenting with phocomelia of the upper limbs, was clinically diagnosed with Rodriguez syndrome (66). Rodriguez syndrome, in which the craniofacial phenotype is similar to that seen in NS and other AFDs, is characterized by the severity of the limb defects, including upper limb phocomelia and lower limb anomalies; most individuals die in fetal life or in the neonatal period due to respiratory distress (66). Because of the initial report of three affected sibs, autosomal recessive inheritance had been assumed for Rodriguez syndrome (68). Apart from the SF3B4-mutated patient described by McPherson et al., the molecular basis of other individuals with Rodriguez syndrome has not been reported, but the findings of McPherson et al. suggest that Nager and Rodriguez syndromes may fall within a spectrum of SF3B4-related disorders. The overlap between MFDGA and NS should also be noted as a patient initially diagnosed as NS with microcephaly was SF3B4 mutation-negative but harbored a heterozygous EFTUD2 mutation (5). Given that microcephaly, one of the major discriminating differences between MFDGA and NS, is occasionally absent in MFDGA patients, EFTUD2 testing may be warranted in SF3B4 mutation-negative Nager patients.

The U2 spliceosomal snRNP contains two major protein complexes, SF3A and SF3B, the latter containing seven proteins (13). Interactions have been identified between SF3B4 (also known as SAP49 or SF3b49) and SF3B2 (also known as SAP145), and between SF3B4 and a region of the pre-mRNA immediately upstream of the intron branch site (69). These studies suggested that the SF3B complex plays a role in tethering the U2 snRNP to the branch site. The yeast ortholog of SF3B4, Hsh49p, is essential for viability and splicing (70). Interestingly, interactions between the SF3B complex and U5 snRNP proteins have been reported, including between SF3B4 and EFTUD2 (71). Hegele et al. have suggested that these SF3B-U5 interactions may be involved in recruitment of the tri-snRNP (U4/U6-U5) by U2 to the spliceosome (71). Non-canonical functions reported for SF3B4 are binding to the bone morphogenetic protein (BMP) receptor BMPR1A and
inhibition of BMP-mediated osteochondral cell differentiation (72, 73). It has been suggested that disruption of this activity of SF3B4 may account for some of the defects in NS (5), although the physiological significance of the SF3B4-BMPR1A interaction remains to be confirmed.

**Cerebrocostomandibular syndrome**

CCMS is a rare disorder characterized by Robin sequence (cleft palate, glossophtosis, and microretornathia) and rib defects such as posterior gaps and missing ribs, frequently leading to death in the early post-natal period due to respiratory distress, while some individuals have been reported with intellectual disability and/or microcephaly (Table 1) (9, 74–76). Familial examples suggestive of both dominant and recessive inheritance have been reported. In 2014, Lynch et al. employed exome sequencing to identify dominant, heterozygous mutations in SNRPB, encoding small nuclear ribonucleoprotein polypeptides B and B1, as a cause of CCMS (9). As discussed above, the major spliceosome consists of the U1, U2, U4/U6 and U5 snRNPs. Each snRNP contains specific protein and RNA components plus a core of seven proteins, known as the Sm proteins, common to all snRNPs. The SNRPB-encoded B and B1 are alternatively spliced versions of one of the seven Sm factors (13). All but one of the SNRPB mutations identified by Lynch et al. fall with an alternative exon containing a premature termination codon (PTC); it had previously been shown that this PTC-containing exon normally serves to auto-regulate SNRPB transcript levels, with inclusion of the exon leading to nonsense-mediated decay (77, 78). In an independent exome sequencing study, Bacrot et al. recently identified de novo mutations in the same alternative exon of SNRPB as Lynch et al., in five CCMS patients (10). The CCMS mutations are clustered at the 5’ and 3’ ends of the PTC-containing exon, in regions known to function as exonic splicing silencers, and the inclusion of this exon was increased in minigene assays using plasmids containing the mutations and in patient cells, elegantly demonstrating that increased NMD of SNRPB transcripts is the likely cause in most individuals with CCMS (9, 10). As noted by Lynch et al., the alternative exon mutations are likely to result in hypomorphic alleles, while complete haploinsufficiency may lead to a phenotype more severe than classic CCMS. Along these lines, it is plausible that a non-exonic regulatory mutation may underlie the cause of CCMS in the one patient studied by Lynch et al. without an exonic SNRPB mutation.

Incomplete penetrance in two families was reported by Lynch et al., while all other SNRPB mutations were de novo or were inherited from an affected parent, suggesting that the previously suspected examples of recessive CCMS in the literature may have been due to dominant but incompletely penetrant SNRPB mutations. In 2009 it was reported that two CCMS-like (MIM 611209) patients harbored the same homozygous variant at the +5 position of intron 5 in COGI, encoding a component of the conserved oligomeric Golgi complex, involved in glycosylation (79). The variant led to exon skipping and a PTC. Of the two patients, only one had posterior rib gaps, which were present only transiently. Whether CCMS is truly genetically heterogeneous remains to be confirmed; it would be of interest to test for SNRPB mutations in the patients described by Zeevaert et al.

 Besides the classic features of CCMS, scoliosis, decreased height, conductive hearing loss and down-slanting palpebral fissures were occasionally present in the SNRPB mutation-positive patients (9, 10), and neurological and cognitive defects were reported in a small proportion of the cohort reported by Lynch et al. Although it had previously been suggested that psychomotor retardation in CCMS may be a secondary effect linked to hypoxia due to respiratory distress at birth, it is also plausible that when present, this phenotype is due to a primary defect in splicing of neurodevelopmental genes.

Finally, it is interesting to note that mutations in SNRPE, encoding another of the seven core Sm proteins, cause a dominant form of isolated hypotrichosis (MIM 615059) (80). This disorder is clinically non-overlapping with CCMS and emphasizes the theme that disruption of different components of the same complex within the spliceosome can lead to vastly different phenotypes.

**Richieri-Costa–Pereira syndrome**

RCPS is a rare AFD involving microstomia, midline cleft of the mandible with micrognathia, absence of lower incisors, cleft palate, laryngeal anomalies, limb reductions (radial, tibial, thumb, thenar, fifth finger and hallux hypoplasia), clubfeet and intellectual disabilities, and has almost exclusively been reported in Brazilian families, consistent with a founder effect (Table 1) (11, 81, 82). Suspecting autosomal recessive inheritance, Favaro et al. performed homozygosity mapping in several affected families and identified segregation of the disease to a 122-kb locus within chromosome 17q25.3 (11). Sequencing of candidate genes in the interval revealed that RCPS-affected individuals have an expansion in the number of copies of an 18–20 nucleotide repeat in the 5′UTR of EIF4A3, inherited in a recessive fashion. The affected individuals harbored 14–16 repeats, whereas healthy controls had 3–12 repeats. In one individual, a missense mutation of a highly conserved amino acid was identified in trans to a repeat expansion allele. EIF4A3 encodes a member of the DEAD-box RNA helicase family, and is a key component of the exon junction complex (EJC), anchoring the EJC to RNA (83). During pre-mRNA splicing, the EJC binds in a sequence-independent fashion, 20–25 nucleotides upstream of spliced exon–exon junctions, where it subsequently serves as a signal involved in nuclear export and cytoplasmic localization of mRNA and regulation of translation and nonsense-mediated decay (83). Recently it has been shown that a spliceosomal factor, CWC22, recruits the EJC to mRNA via direct interaction with EIF4A3, highlighting the link between splicing and EJC deposition (84–86). EIF4A3 is one of the four proteins that make up the core of the
EJC (Fig. 2). Interestingly given the anterior limb deficiencies in RCPS, compound heterozygosity for a null allele and a regulatory non-coding variant in RBM8A, encoding Y14, another of the four EJC core components, causes thrombocytopenia with absent radius syndrome (MIM 274000) (87, 88). It was hypothesized that the pathogenetic mechanism underlying RCPS is partial loss-of-function of EIF4A3; transcript levels in blood and mesenchymal cells were 30–40% lower in RCPS individuals compared to controls (11). All RCPS patients studied by Favaro et al. and harboring EIF4A3 mutant alleles were Brazilian. It will be of great interest to identify further mutant alleles of EIF4A3, although those leading to complete loss-of-function (when recessive) may not be compatible with life. Patients with milder phenotypes, presenting just one of the clinical hallmarks of the syndrome, such as cleft mandible or altered larynx, could also be screened for variants in EIF4A3.

**Burn-McKeown syndrome**

BMKS is a rare MFD characterized by choanal atresia, hearing loss and recognizable facial features that include cleft lip and/or palate, coloboma of the lower eyelid, short palpebral fissures and prominent nasal bridge, occasionally associated with visceral malformations such as congenital heart defects (Table 1) (12). All patients have normal intellectual development; TCS is therefore a possible differential diagnosis. To date, less than 20 individuals have been reported, including a large consanguineous Alaskan family initially reported as having oculo-oto-facial dysplasia (12, 89–92). In 2014, with an approach using microarray analysis, MLPA, exome sequencing and whole genome sequencing, Wieczorek et al. reported TXNL4A as the causative gene for BMKS (12). Microarray and MLPA revealed heterozygous microdeletions encompassing TXNL4A in five families, whereas heterozygous loss-of-function mutations in three remaining families were found via exome sequencing. Two deletions and all the mutations were inherited from a healthy parent; parental DNA was not available for the three other patients with a deletion. Because haploinsufficiency was unlikely to be the causative mechanism, whole genome sequencing was performed in order to identify non-coding variants contributing to the phenotype in BMKS patients. The same heterozygous 34 base pair (bp) deletion (type 1 deletion) in the promoter region of TXNL4A was identified in eight families; in each family tested it was inherited from the healthy parent without the null mutation (12). The affected individuals of the consanguineous Alaskan family were homozygous for a different 34 bp deletion (type 2 deletion) in the same region of the promoter. Luciferase reporter assays demonstrated a reduction of reporter expression driven by the promoter harboring the deletions, with a more severe reduction for the type 2 deletion, compared to the wildtype promoter (12). Interestingly, sequencing of control samples indicated a predicted frequency of 1 in 17,300 for homozygous type 1 deletion carriers, and one homozygous type 1 individual was found, without phenotypic information. Given this frequency, it is probable that the type 1 promoter deletions are not full loss-of-function alleles; it will be interesting to study the phenotypic severity in a larger number of individuals homozygous for TXNL4A promoter deletions. At the other extreme, homozygous or compound heterozygous null mutations may lead to a more severe phenotype than BMKS. Therefore, the findings of Wieczorek et al. suggest that the BMKS phenotype in most instances is the result of a specific dosage of TXNL4A, i.e. the outcome of one null allele plus reduced transcription of the remaining allele.

The yeast ortholog of TXNL4A, DIB1, encodes a core component of the U5 snRNP, with DIB1 mutants defective in assembly of the tri-snRNP (12) and consequently in pre-mRNA splicing (93). Therefore, of the eight core proteins of the U5 snRNP, five are now known to be mutated in human disease – TXNL4A in BMKS, EFTUD2 in MFDGA, and PRPF6, PRPF8 and SNRNP200 in retinitis pigmentosa.

**Perspectives**

Here we have reviewed five syndromes with major craniofacial involvement whose etiology involves defects of spliceosomal function or of associated events during mRNA processing. Surprisingly, despite the widespread importance of mRNA splicing in eukaryotic cells, these syndromes each have a relatively restricted range of phenotypes. The craniofacial involvement is typically severe, and anterior limb and/or other skeletal defects are frequently present. Intellectual disability and extracranial malformations are inconstant. Of note, two other MFDs, TCS and DBA-MFD, are caused by ribosomal dysfunction. Similar to the observations made for the spliceosomopathies described here, haploinsufficiency for ubiquitously-expressed genes involved in ribosome biogenesis or function can lead to tissue-specific diseases such as Diamond-Blackfan anemia (MIM 105650) (94), Shwachman-Diamond syndrome (MIM 260400) (95) or isolated congenital asplenia (MIM 271400) (96). It is possible that this is in part due to differential rates of growth (and hence of requirements for protein production) across tissue types during embryonic development. It has also been suggested that, rather than the ribosome behaving as a ‘monolith,’ the expression or activity of certain ribosomal components may differ in different cell types, with the consequence being cell type-specific distributions of translated mRNAs (97, 98). Perhaps cell type-specificity of spliceosomal function is achieved in a similar way. To this end, it would be of interest to systematically compare the expression profiles of the five genes discussed in this review with each other and with genes encoding other spliceosomal components, in disease-relevant tissues during normal development. Furthermore, for the spliceosomal genes described in this review, transcriptomic profiling of cells from patients with mutations in these genes, or of developing craniofacial tissue from mouse mutants, will enable investigation of whether certain genes and pathways are particularly prone to splicing defects associated with any given mutation.
Craniofacial disorders and the spliceosome


Craniofacial disorders and the spliceosome