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Mutations in U4atac snRNA, a Component of the Minor Spliceosome, in the Developmental Disorder MOPD I

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Abstract

Small nuclear RNAs (snRNAs) are essential factors in mRNA splicing. By homozygosity mapping and deep sequencing, we show that a gene encoding U4atac snRNA, a component of the minor U12-dependent spliceosome, is mutated in individuals with microcephalic osteodysplastic primordial dwarfism type I (MOPD I), a severe developmental disorder characterized by extreme intrauterine growth retardation and multiple organ abnormalities. Functional assays show that mutations (30G>A, 51G>A, 55G>A, and 111G>A) associated with MOPD I cause defective U12-dependent splicing. Endogenous U12-dependent but not U2-dependent introns are poorly spliced in MOPD I patient fibroblast cells while introduction of wild type U4atac snRNA into MOPD I cells enhances U12-dependent splicing. These results illustrate the critical role of minor intron splicing in human development.

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Keywords

microcephalic osteodysplastic primordial dwarfism type I; *RNU4ATAC*; mutation; splicing; snRNA; minor spliceosome

U4atac snRNA is a component of the minor spliceosome, and is required for the proper excision of the U12-dependent class of introns (1-4). While accounting for only about 800 introns in humans, U12-dependent introns are found in many essential genes (e.g. DNA replication and repair, transcription, RNA processing and translation) (5). Thus, mutations in an snRNA required to splice such introns are likely deleterious and presumably would result in significant developmental or clinical consequences.

U4atac snRNA appears to be encoded by a single gene (*RNU4ATAC*) on chromosome 2q14.2. Here we report that biallelic mutations in this gene are found in a severe developmental disorder, MOPD I (also known as Taybi-Linder syndrome, OMIM 210710) (6). The main features of MOPD I patients are extreme intrauterine growth retardation, abnormalities in multiple organs, and death in infancy or early childhood (7, 8). We focused our initial studies on cases diagnosed in the Amish of Ohio (9) (fig. S1), where uniform phenotypic features and a high degree of consanguinity suggested the existence of a single founder mutation. Briefly, we applied genome-wide homozygosity mapping followed by targeted, high-throughput second-generation sequencing in search of mutations at chromosome 2q14.2 (Fig. 1, fig. S2-S4). A novel g.51G>A variant within the non-protein coding *RNU4ATAC* gene was detected in homozygosity in all 7 Amish patients studied and in heterozygosity in 13 Amish parents. An Australian patient had the same mutation whereas in 2 German MOPD1 families, biallelic g.55G>A occurred in one patient and compound heterozygous g.30G>A and g.111G>A occurred in another patient. The 51G>A mutation represents a founder event in the Amish, as shown by haplotype analysis (fig. S3 and S5). This mutation was found in 16/281 Ohio Amish controls but in none of 180 Pennsylvania Amish controls. It was also seen in 2/720 controls from central Ohio but not in 370 controls from France. The three mutations found in German MOPD1 families were not found in 452 central Ohio controls. We conclude that the genetic findings are fully compatible with the expected recessive inheritance of rare mutations in the same gene. Further details of the mapping, sequencing, mutation analyses and haplotyping are in the SOM.

The 30G>A, 51G>A, and 55G>A mutations in the U4atac snRNA are located within an important structural feature known as the 5′ stem-loop, while the 111G>A mutation is located in another essential stem region, the 3′ stem-loop (10). These mutations are predicted to disrupt the snRNA's secondary structure and cause defects in the minor spliceosome (fig. S6) (11-13). To evaluate functional effects of these mutations, we assayed the *in vivo* splicing of a modified U12-dependent intron reporter whose splicing is dependent on expression of a modified, exogenous U4atac snRNA, denoted as U4atac-ATH (figs. S7 and S8) (10). Compared with the wild type U4atac, each of the MOPD I mutations in U4atac snRNA reduce U12-dependent splicing activity by greater than 90% (Fig. 2A). This suggests that all four mutations cause significant defects in U12-dependent splicing. Most of the splicing defect of the 51G>A mutation could be rescued by combining it with the 32C>T mutation in the presumed base pairing partner (Fig. 2A lane 8 and fig. S6). This suggests that the MOPD I mutation abrogates U4atac snRNA function by disrupting the RNA secondary structure.

U12-dependent introns are conserved in a variety of gene families implicated in different physiological processes (5, 14). To assess U12-dependent splicing in MOPD I cells, we compared fibroblasts obtained from two MOPD I patients (with the 51G>A mutation) and

two normal human fibroblast cell lines. Using real time RT-PCR to quantify the levels of spliced and unspliced U12-dependent and U2-dependent introns, we found that all examined U12-dependent introns were less efficiently spliced in the MOPD I cells while U2-dependent introns were not significantly affected (Fig. 2B). Expression of wild type U4atac snRNA in the MOPD I cells increased splicing of U12-dependent introns while having little effect on U2-dependent introns (Fig. 2C). Note that intron 5 and 12 which are the most affected (Fig. 2B) also show the greatest increase upon restoration of wild type U4atac snRNA (Fig. 2C). We also observe considerable variability in the reduction of splicing of individual U12-dependent introns in patient cells (Fig. 2B). Such variability may underlie the specific developmental defects seen in MOPD I. Similar variability has been observed in *Drosophila* carrying a mutation in U6atac snRNA (15).

These results are fully compatible with the idea that the MOPD I mutations in U4atac snRNA reduce *in vivo* splicing of U12-dependent introns with potentially deleterious consequences for proper levels of gene expression and/or alternative splicing. Our findings illustrate the critical role of the minor spliceosome in human development. Future work to define the downstream affected genes in MOPD I patients is warranted.

Several observations suggest that normal function of the minor spliceosome is crucial for viability and development (16, 17). Other human diseases caused by mutations in protein components of small nuclear ribonucleoprotein (snRNP) complexes that contribute to spliceosome functions have been described (18, 19). We found genetic mutations in the U4atac snRNA gene by high throughput sequencing of the entire mapped genomic locus, notably including non-protein coding DNA. Much of the current emphasis in finding the mutations underlying Mendelian disorders is focused on exome sequencing which would not have found the MOPD I mutations. This illustrates the need to sequence the genome rather than the exome in many situations.

Establishing a clinical diagnosis of MOPD I and III as well as MOPD II, Seckel syndrome and related disorders has been difficult until now, given a significant overlap in clinical features (8, 20). In one study, mutations in the pericentrin *(PCNT)* gene were reported in patients with MOPD II while *PCNT* mutations were not seen in MOPD I or III, Seckel syndrome or unclassified growth retardation (21). By contrast, other studies showed mutations in *PCNT* in patients with Seckel syndrome or MOPD II (22-24). Even among the cases reported here, there were significant differences in the phenotypic features and lifespans. We anticipate that further characterization of distinct mutations in *RNU4ATAC* will shed light on some of these difficulties of clinical classification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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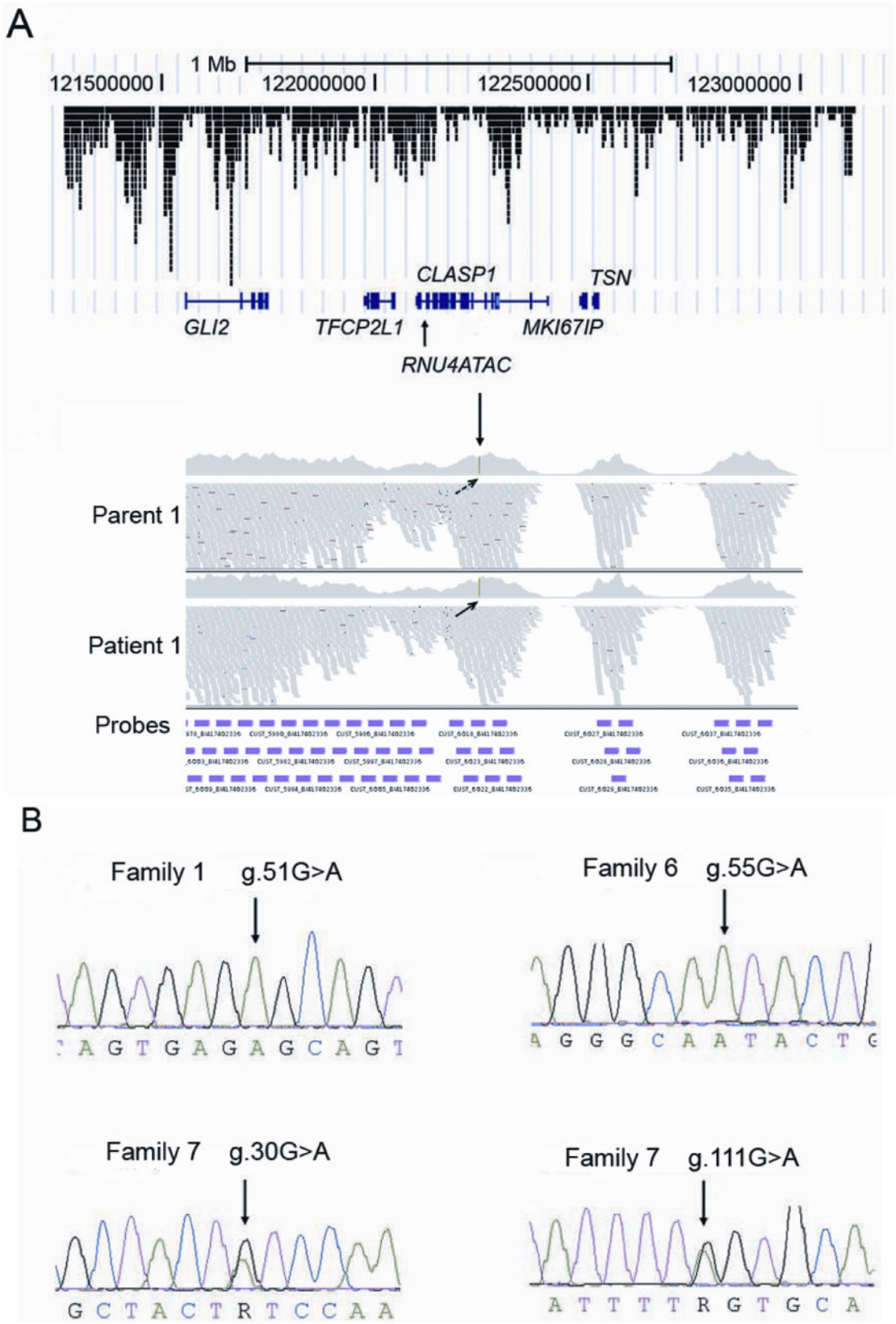
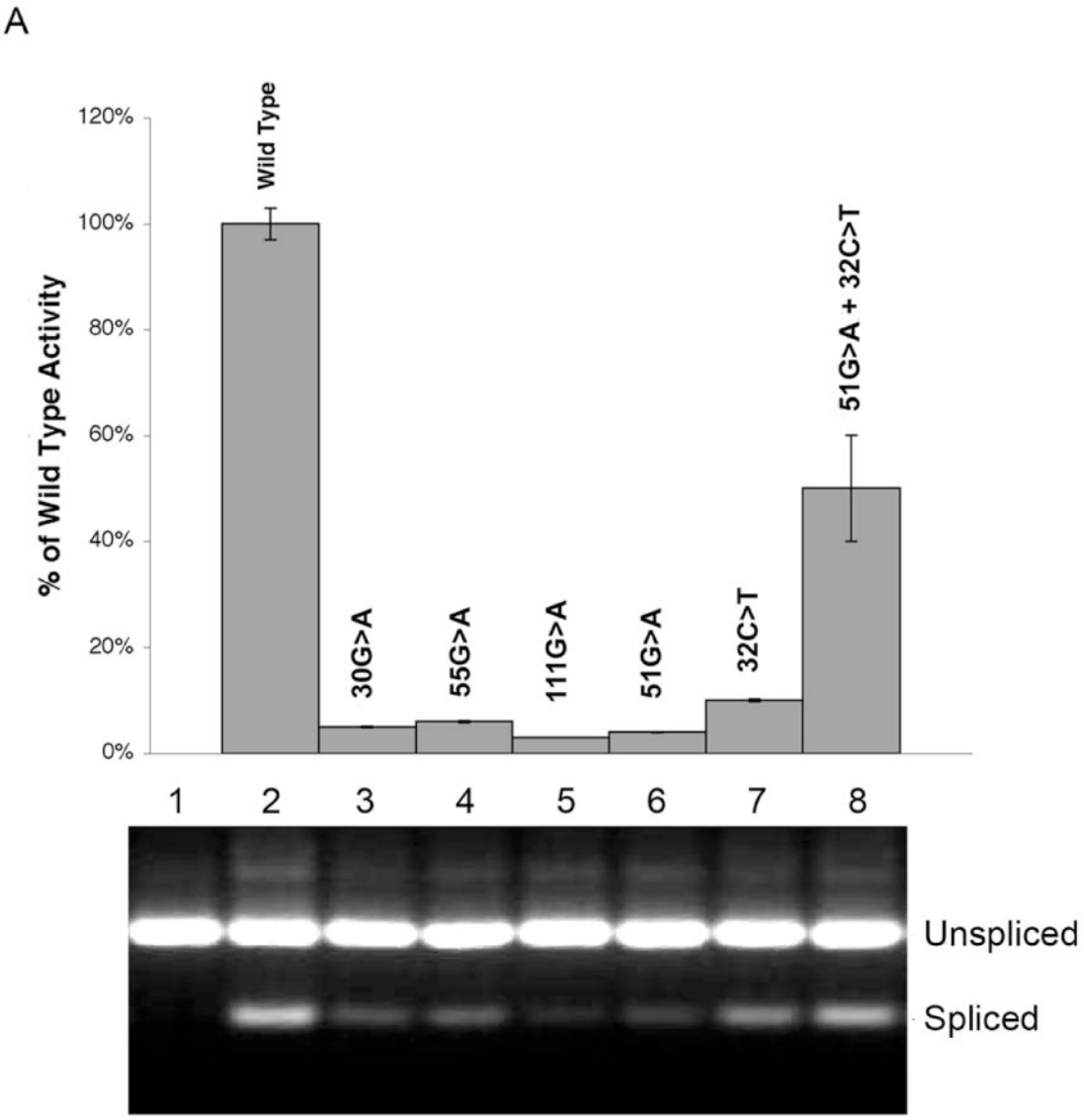


Fig. 1.

Identification of *RNU4ATAC* mutations in MOPD I patients. (A) High throughput sequencing of ~1.8 Mb mapped region. *Top:* Overview of enriched sequence reads from a single MOPD I patient, mapped to the target region showing chromosome 2 coordinates and schematic of genes. *Bottom:* Paired end reads mapped to targeted region, visualized using IGV browser (Broad Institute), showing the depth of sequencing coverage (*gray peaks*) at tiled enrichment probes (*purple rectangles, bottom*); see Supplementary Table 1. Very few sequence reads are mapped beyond 200 nt into regions lacking enrichment probes. *Dotted arrow*: heterozygous mutation in parent 1; *solid arrow*: homozygous mutation in patient 1. (B) Conventional Sanger sequencing chromatograms show the distinct homozygous mutations (51G>A; 55G>A) and compound heterozygous mutations (30G>A and 111G>A) in MOPD I patients.



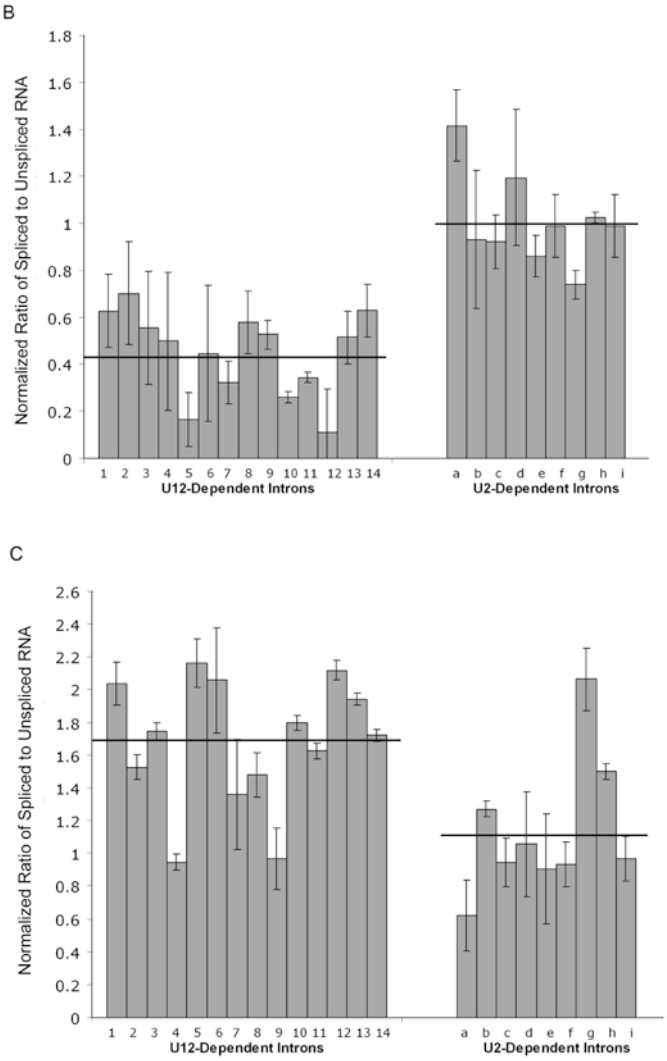


Fig. 2.

Mutations in *RNU4ATAC* affect U12-dependent spliceosomal function. (A) U4atac snRNA mutations found in MOPD I patients disrupt minor class intron splicing *in vivo*. Chinese Hamster Ovary cells were transfected with a test intron and various snRNA constructs (fig. S8) (10). After 48 hr, RNA splicing products were analyzed by RT-PCR, followed by agarose gel electrophoresis to determine U12-dependent minor spliceosome activities. Effects on splicing *in vivo* were quantitated relative to the wild type U4atac titration curve shown in figure S9 and plotted as the mean and standard deviation of triplicate transfections. Lane 1 contains no U4atac snRNA construct while lanes 2-8 contain U4atac-ATH constructs with the indicated sequence changes. (B) Endogenous U12-dependent introns are inefficiently spliced in MOPD I cells. The ratio of spliced to unspliced pre-mRNA for U12- and U2-dependent introns was determined using real time RT-PCR. Two MOPD I fibroblast cell lines homozygous for the 51G>A mutation were compared to two normal fibroblast cell lines. The spliced to unspliced ratio in the normal cells was set to unity for each intron. The horizontal lines show the group average for U12-dependent introns (average=0.45) and U2-dependent introns (average=1.01). The genes and introns examined are listed in table S2. (C) Restoration of wild type U4atac snRNA increases U12-dependent splicing in MOPD I cells. MOPD I fibroblasts were transfected with a wild type human U4atac snRNA gene driven by a U1 snRNA promoter or vector DNA alone. The same introns were measured for splicing

as in panel B. The spliced to unspliced ratio of the cells transfected with vector alone was set to unity. The horizontal lines show the group average for U12-dependent introns (average=1.67) and U2-dependent introns (average=1.14).