An Intragenic Deletion of the Gene MNAT1 in a Family with Pectus Deformities

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Pectus carinatum and excavatum have multiple genetic associations. We report on a novel association of these deformities in a 34-month-old male and his father, likely due to a small intragenic deletion of MNAT1 (ménage a trois 1 gene). Both individuals share a deletion of MNAT1 located at 14q23.1 and an interstitial duplication of CHRNA7 located at 15q13.3. Deletion of MNAT1 has been associated with connective tissue abnormalities and is likely the etiology of the malformations, whereas the duplication of CHRNA7 is unlikely related due to the lack of association with any such connective tissue abnormalities.

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Key words: pectus carinatum and excavatum; 14q23.1 microdeletion syndrome; MNAT1 gene

INTRODUCTION

Pectus Carinatum (PC) and Pectus Excavatum (PE) are uncommon skeletal anomalies, where the sternum arches and the costal cartilages are depressed inward (PC) or displaced outward (PE) [Robicsek and Watts, 2010]. The prominence is progressive, and can be accompanied by cardiorespiratory complications, including dyspnea, wheezing, and exercise intolerance [Coelho and Guimaraes, 2007]. As such, surgical repair is often performed for cosmetic improvement and to alleviate or prevent cardiopulmonary difficulties [Robicsek and Watts, 2010].

PC and PE make up approximately 90–97% of all chest wall deformites [Brochhausen et al., 2012], although PE is 3–13 times more common [Coelho and Guimaraes, 2007]. PC may also have an up to 5:1 male:female predominance, and appears more frequently in Hispanic and Caucasian ethnic groups [Fonkalsrud, 2008].

PC or PE is thought to have an underlying genetic and/or biomechanical etiology, though the mechanism is debatable [Coelho and Guimaraes, 2007; Kotzot and Schwabegger, 2009]. A proposed biomechanical mechanism is altered cartilage organization and distribution [Feng et al., 2001; Brochhausen et al., 2012]. Pectus deformity is associated with various conditions, including syndromes, disruption sequences, and genetic associations [Kotzot and Schwabegger, 2009]. Heterogeneity of PC and PE are illustrated by their various syndrome associations as they are found in Marfan Disease, Osteogenesis Imperfecta (types I, III, IV), Cardiacofacial cutaneous syndrome, Morquio syndrome, prune belly syndrome, Noonan syndrome, mitral valve stenosis or prolapse, hand agenesis, Poland anomaly, Moebius anomaly, Holt-Oram syndrome, Pentalogy of Cantrell, and PHACE [Kotzot and Schwabegger, 2009; Robicsek and Watts, 2010]. Inheritance of isolated pectus deformity may be autosomal dominant (OMIM 609143, 600399) or autosomal recessive (as reported in a case report of a brother and sister with facial anomalies and pectus carinatum) [Kotzot and Schwabegger, 2009]. We report here on a novel intragenic deletion of MNAT1 (ménage a trois 1 gene, OMIM #602659) located at 14q23 found in father and child with pectus deformities.

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Macrocephaly was noted at 7 months. He was then noted to have developmental delay and language regression starting at 16 months. MRI from that time was normal. He had an adenoidectomy at 25 months, and then was formally evaluated and diagnosed with both profound language delay and autism at 27 months. Urine organic acids, CMP, EEG, and fragile X panel were negative from that assessment.

The patient's physical exam was significant for macrocephaly (at 100th centile for age) with a broad appearing face. He had a down turned nasal tip, open mouth with widely spaced teeth, and an appropriate philtrum (Fig. 1). He wore glasses for strabismus. He had a prominent pectus carinatum (Fig. 1), broad and shortened fifth digits bilaterally and second/third toe syndactyly bilaterally. His neurological exam was grossly normal. His father had a notable pectus excavatum (Fig. 1) and reported a history of learning disability as a child. The patient's twin sister reportedly had mild developmental delay but no unusual physical phenotype. The patient’s mother was healthy and had no thorax anomaly.

An a-CGH (array-comparative genome hybridization) using NimbleGen CGX-12/hg-18 (Roche, Madison, WI) slide and genoglyphix software analysis (Signature Genomics, Perkin Elmer, Spokane, WA) of the patient and father’s DNA revealed an interstitial deletion and duplication in the DNA of the peripheral blood specimen: arr[hg18]14q23.1(60,326,513–60,438,900) × 1, 15q13.3 (29,816,893–30,226,405) × 2.

The deletion is characterized by a copy loss of seven oligonucleotide probe(s) in the region of 14q23.1 (Fig. 2). This abnormality size is 112.39 kb and involves part of the gene MNAT1 (ménage a trois 1 gene, OMIM# 602659). Based on a reference sequence of the gene made of eight exons, the deletion is intragenic including six exons (Fig. 2). The interstitial duplication is characterized by a copy gain of 58 oligonucleotide probe(s) in the region of 15q13. It is 409.51 kb and involves the OMIM gene CHRNA7 (Fig. 2). Paternal a-CGH revealed an identical 14q23.1 deletion and 15q13.3 duplication; therefore both abnormalities are paternally inherited.

**DISCUSSION**

The etiology of PE and PC remains unknown. The deformities could be the result of a genetic defect (as in Noonan Syndrome) [Kotzot and Schwabegger, 2009] or an environmental factor (such as a surgical complication of PE repair) [Robicsek and Watts, 2010]. The above reported father and child that exhibit PE and PC, respectively, have two genetic abnormalities: deletion of MNAT1 and duplication of CHRNA7.

The duplication at 15q13.3 involves the OMIM gene CHRNA7, which is found in normal controls as reported by the Database of Genome Variants (DGV; http://dgv.tcag.ca/) but also acts as an inherited genetic modifier and subunit of the nicotinic acetylcholine receptor [Tassan et al., 1995]. This gene is thought to contribute to epilepsy if deleted [Helbig et al., 2009], and may

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**FIG. 1.** A: The patient's profile demonstrating broad appearing features including down turned nasal tip and open mouth with widely spaced teeth; B) Lateral view of the patient's chest demonstrating pectus carinatum; C) Anteroposterior view of the chest of the patient's father demonstrating pectus excavatum; and D) Facial profiles of the patient and his father together demonstrating similar facial structures (broad cheeks, down turned nasal tip).
FIG. 2. A: The interstitial deletion of 14q 23.1 with the following genomic coordinations: arr[hg18]14q23.1(60,326,513–60,438,900) × 1 which involves part of MNAT1 including all the exons as depicted by the genome browser UCSC plot. B: The interstitial duplication of 15q 13.13 with the following genomic coordinations: arr[hg18] 15q13.3(29,816,893–30,226,405) × 3 which involves part of CHRNA7 as depicted by the genome browser UCSC plot.
The intragenic deletion of \textit{MNAT1} has never been reported as a sole anomaly, nor found in the normal population in the database of genomic variants [http://dgv.tcag.ca]. \textit{MNAT1} gene produces the protein \textit{MAT1} (ménage à trois 1), which is a RING finger protein [Tassan et al., 1995]. \textit{MAT1} is one of the three proteins that form mammalian \textit{CDK}-activating kinase (CAK), which in turn activates cyclin bound \textit{CDK}s (cyclin-dependent kinases) via phosphorylation at a specific threonine residue in the \textit{CDK}s’ T-loop [Eki et al., 1998]. \textit{MAT1} specifically forms complexes with \textit{CDK}7 and cyclin H to form the multisubunit protein complex \textit{TFIHH} [Eki et al., 1998]. This complex is a requirement for RNA polymerase II-catalyzed transcription and DNA nucleotide excision repair, which modulates cell cycle control [Eki et al., 1998; Talukder et al., 2003]. \textit{MAT1} appears necessary for stabilization of the complex and appears to facilitate its assembly [Rossi et al., 2001; Korisaaari et al., 2002]. This three-member complex has been demonstrated in vivo in all parts of the cell cycle [Tassan et al., 1995]. In humans, \textit{TFIHH} has additionally been shown to interact with \textit{MAT1} (metastasis associated protein (1) to regulate estrogen receptor transactivation and interact with \textit{a} and \textit{g} retinoic acid receptors [Korisaaari et al., 2002; Talukder et al., 2003]. Overall, \textit{MAT1} is necessary for cell cycle progression, basal transcription, and DNA repair [Tassan et al., 1995; Eki et al., 1998].

Furthermore, a knock-out mouse model of \textit{MNAT1} showed a clinically relevant phenotype in a Cre/LoxP system [Korisaaari et al., 2002]. Mouse \textit{Mat1} appeared to have absolute necessity in the sustained vitality of some cell lines (i.e., germ lines), but appears more regulatory in other cell lines (i.e., Schwann cells) [Korisaaari et al., 2002]. Homozygous deletion of this gene created a phenotype in mice where they were completely devoid of germ cells by 10 weeks of age, had abnormal gait after 3 months of age, were affected with neurogenic muscular atrophy at 3 months of age, and were affected with demyelination of nerve cells by 5 months of age [Korisaaari et al., 2002]. The mechanism of demyelination is not determined by this study; however, it has been elsewhere described that collegen \textit{a4(V)} is necessary for Schwann cell myelination [Chernousov et al., 2006]. Related, a proposed cellular mechanism of pectus excavatum is disorderly arrangement and distribution of collagen II in cartilage [Feng et al., 2001]. Although the patient deletion is monoallelic, a mutation of the second allele cannot be ruled out by the resolution of the present study. However, the impact of knock-out gene in mice is not always equivalent to homozygous deletion in human. It is plausible that there is a related mechanism through which \textit{MAT1} causes acquired collagen dysregulation in both instances.

Heterozygous deletion of human \textit{MAT1} as reported here may cause a subtle connective tissue malfunction late in development that could lead to the demonstrated pectus phenotypes. Studies by Rossi et al. [2001] demonstrated that heterozygous \textit{Mat1}+/− mice were genotypically equivalent to \textit{Mat1}+/+/ mice, though the phenotype of these mice beyond trophotoderm cells was not commented on. This could be considered consistent with later appearing phenotypic changes in the mentioned studies by Korisaaari et al. Furthermore, embryonic lethality in \textit{Mat1}−/− mice was attributed to depletion of the maternal \textit{Mat1} present at fertilization [Rossi et al., 2001]. It is unreported what amount of active protein is necessary for normal cell function or whether decreased protein activity results in progressive phenotypic changes later in differentiation.

Seckel syndrome is known to be associated with DNA repair mutation. Four gene loci are mapped to this syndrome: \textit{SCKL1} at 3q22.1–q24, \textit{SCKL2} at 18p11.31–q11.2, \textit{LIG4} at 13q33.3 and \textit{SCKL3} at 14q23 [Kilinct et al., 2003]. \textit{MNAT1} gene was considered to be the best candidate to be responsible for \textit{SCKL3} as identified by linkage analysis in a group of five families of Turkish descent [Kilinct et al., 2003]. The syndrome is an autosomal recessive syndrome characterized by microcephaly, a “bird-like” head, cleft palate, dentition abnormalities, pancytopenia, and ocular manifestations [Kilinct et al., 2003]. None of these features are found in our patient; however, the phenotype of this syndrome varies tremendously due to genetic heterogeneity [Kilinct et al., 2003]. One patient, a 12-year-old female, has phenotype that includes pectus carinatum [Kilinct et al., 2003]. Although most patients reported had a homozygous genotype, one affected brother had a heterozygous genotype, raising the question whether one deleted copy of \textit{MNAT1} is haploinsufficient causing a milder phenotype like is found in the present family.

Additionally, Holt-Oram syndrome is characterized with upper limb, chest wall, and heart deformities, which includes pectus deformity. It has two mapped loci [Kotzot and Schwabegger, 2009]: 12q21–24, in which \textit{TBX5} is thought to play a role in pectus deformity [Boogerd et al., 2010]; and 14q23–24 [Turleau et al., 1984], in which an undetermined gene is involved. \textit{MNAT1} may be involved in pectus deformity when it is caused by the second locus.

Similarly, a large interstitial deletion of 14q23 encompassing seven genes including \textit{MNAT1} was associated with increased risk of intracranial aneurysm in a Japanese population [Mineharu et al., 2008]. The connective tissue disorganization that is responsible for this population’s significant variation may also be an etiology of cartilage defects and pectus deformity. Related, a large duplication of a similar region that included \textit{MNAT1} (14q22.3–q23.3) was associated with bronchial anomalies, deafness and preauricular pits [Nolen et al., 2006]. This could plausibly be part of a connective tissue disorder linked to cartilage dysregulation.

In conclusion, the patient’s paternally inherited intragenic deletion of \textit{MNAT1}, involving the internal six exons of the genes, is plausibly a mechanism of pectus deformities. Mutation analysis of \textit{MNAT1} gene in individuals with sole feature of PC or PE and additional reported clinical cases with a similar size deletion are needed to confirm this unique and novel association.
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