Original Article

Compound heterozygous mutations in TTC7A cause familial multiple intestinal atresias and severe combined immunodeficiency


Familial multiple intestinal atresias is an autosomal recessive disease with or without combined immunodeficiency. In the last year, several reports have described mutations in the gene TTC7A as causal to the disease in different populations. However, exact correlation between different genotypes and various phenotypes are not clear. In this study, we report identification of novel compound heterozygous mutations in TTC7A gene in a Malay girl with familial multiple intestinal atresias and severe combined immunodeficiency (MIA-SCID) by whole exome sequencing. We found two mutations in TTC7A: one that destroyed a putative splicing acceptor at the junction of intron 17/exon 18 and one that introduced a stop codon that would truncate the last two amino acids of the encoded protein. Reviewing the recent reports on TTC7A mutations reveals correlation between the position and nature of the mutations with patient survival and clinical manifestations. Examination of public databases also suggests carrier status for healthy individuals, making a case for population screening on this gene, especially in populations with suspected frequent founder mutations.

Conflicts of interest

The authors have no competing financial interests or conflicts of interest to declare.

Familial multiple intestinal atresias (MIA) (MIM [243150]) is a very rare and severe congenital disease, which often involves a variable combination of sites from stomach to rectum. It was first reported in the early 1970s (1, 2) and was proposed as an autosomal recessive disorder. Although most cases described were sporadic, multiple affected siblings in a family were also described, and sometimes from consanguineous marriages (3, 4), suggesting recessive inheritance nature of the disease.

In addition to multiple intestinal atresias, severe immunological defect and sepsis were often reported in these patients. In 1990, Moreno et al. described the first association of MIA and immunodeficiency (3). In 1998, Lambrecht and Kluth reviewed 35 cases of MIA, five of whom were reported to have immunodeficiency (5). Persistent T and B cell lymphocytopenia and very low immunoglobulin were often observed (6, 7).

The genetic causes of the disease have remained elusive until recently with advances in next generation-
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sequencing such as whole exome sequencing (WES). In 2013, Samuels et al. examined a total of seven families with MIA, by WES in some families and followed up with candidate gene screening on the others. In all seven families, a four base pair intronic deletion in TTC7A (tetratricopeptide repeat domain 7A, MIM 609332) was detected. This mutation was immediately downstream of the consensus splice donor GT and was shown to cause skipping of exon 7 during splicing, causing a shift of the open reading frame. For six of the seven families, homozygous 4-bp deletion was detected while for the seventh family, an additional missense mutation (p.L823P) was detected, forming compound heterozygous with the 4-bp splicing junction deletion. The first six families were all of French-Canadian descent and the seventh family had French-Canadian ancestry on one side of the family and English ancestry on the other (8). Interestingly, among the families described by Samuels et al. only the patient in family 7 showed severe immunodeficiency, characterized by recurrent infections associated with hypogammaglobulinemia and profound T cell lymphopenia.

Shortly after, Chen et al. identified TTC7A mutations in five patients from unrelated families with SCID-MIA using WES and in three additional patients using a candidate gene approach. They showed that TTC7A is expressed in human thymus through immunohistochemistry. Severe lymphoid depletion in the thymus and peripheral lymphoid tissue in two of the affected patients was also observed. Unlike the cases reported by Samuel et al. all of their patients showed various levels of T-cell lymphopenia. In particular, profound CD8 T-cell lymphopenia was found in all of their patients.

All the six unrelated MIA-CID patients described by Bigorgne et al. showed profound, generalized lymphocytopenia and milder natural killer (NK) and B cell lymphopenia. Thymus hypoplascity and abnormal distribution of epithelial cells were also observed. Patients showed profound disruption of the epithelial barrier along the gastrointestinal tract. Inversion of apicobasal polarity of the epithelial cells was observed, which can be reversed by inhibition of Rho kinase (9). The patients showed pseudostratified gut epithelium, disturbed nuclear positioning and signs of apoptosis in the epithelium.

Avitzur et al. performed WES on a patient presented with severe exfoliative apoptotic enterocolitis (AE) and carried out candidate gene screening in 40 pediatric patients with very early onset inflammatory bowel disease (VEOIBD). They detected TTC7A mutations from this patient and two other unrelated families. Interestingly, patients from two families, Family 1 and Family 3 (referred to as A_F1 and A_F3 here, respectively), suffered from apoptotic enterocolitis but had no evidence of MIA or stricturing disease upon autopsy, quite different from the previous three reports on TTC7A mutations. Family 3 also did not show overt defect in T cells.

Although the causal nature of TTC7A to MIA-CID or MIA-SCID has been established through these findings and functional studies, questions remain on the exact correlation between mutations in this gene and phenotypic variations. Because the majority of the reported mutations were nonsense mutations or frameshift insertion/deletions (indels) causing total abolition of the protein function, it is reasonable to predict that many missense mutations in this gene may exist and show various and likely hypomorphic manifestations that are yet to be identified. Here we review the findings on TTC7A reported so far and also report a novel case of MIA-SCID with two previously unreported compound heterozygous mutations in TTC7A. The founder nature of some of these mutations, and the existence of potentially deleterious mutations found in public databases in apparently healthy individuals are also discussed.

Subjects and methods

Clinical data

The proband was a Malay girl first visited the Division of Paediatric Surgery & Paediatric Urology at the University of Malaya and then was referred to the Department of Paediatrics of the same university at 4 months of age. She had a history of MIA that involved the pyloric, duodenal, jejunal, ileal and colonic segments. Antenatal ultrasound at 7 months of gestation showed polyhydramnios and MIA. She was the second child of a non-consanguineous family. Her mother also had a history of two previous spontaneous miscarriages (first and third pregnancy, Fig. S1, Supporting Information). The patient’s older brother was also diagnosed with MIA and died 3 days after birth of post-operation complication consisting of severe septicemia and multiple organ failure.

The patient was delivered at term, via elective cesarean with a birth weight of 2.2 kg. Postnatally, she underwent three laparotomies. On day 2 of life, she had the first laparotomy and ileostomy which included the removal of the atretic bowel. This was complicated by severe gastro-oesophageal reflux, which required a second and third laparotomy. She developed short gut syndrome with just 45 cm of bowel remaining. On physical examination, her weight was below the third percentile while her length was at the 25–50th percentile, and occipito-frontal circumference was at the 50th percentile. She was not dysmorphic. Other physical examination was unremarkable with no hepatosplenomegaly and the presence of an ileostomy stoma in situ was noted. The histopathological report stated the small bowel lumen was occluded by fibrous tissue and a fenestrated ‘sieve-like’ atresia was noted – the latter formed by intestinal mucosa and lamina propria while the muscularis was deficient (Fig. 1).

The proband’s lymphocyte subset and immunoglobulin levels are presented in Table 1. Cytogenetic studies including karyotyping and Fluorescence in situ Hybridization (FISH) were normal. A diagnosis of multiple intestinal atresias with severe combined immunodeficiency was made. The patient received total parenteral nutrition and supportive care. Unfortunately the patient died of septicemia at 27 months of life during the preparation of this manuscript. The parents gave informed consent for genetic studies for this case and the study has
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Whole exome sequencing (WES)

WES was performed for the index patient and her parents, using Illumina HiSeq 2000 (Illumina, Inc., San Diego, CA 92122 USA) on genomic DNA enriched for exonic fragments using Agilent SureSelect V3 (Agilent technologies, Santa Clara, CA 95051 USA). The data were processed using GATK (10). Briefly, paired-end reads were aligned to human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner (11). Picard was used to mark duplicated reads and RealignerTargetCreator and BaseRecalibrator of GATK were used for realignment and base quality recalibration. Variants were considered as low quality and were removed if they had low coverage depths (<10x), low mapping quality (<20, Phred scale), or low variant quality (<30, Phred scale). Mutations were annotated using ANNOVAR (12). Population variant allele frequencies from Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS/), the 1000 Genomes project variant dataset and an internal dataset on WES data from approximately 200 individuals from unrelated studies conducted in the Department of Paediatrics and Adolescent Medicine of the University of Hong Kong were used to filter out the common variants (>3% from any of these databases) that are considered unlikely to be causal mutations for this case.

Evaluation of protein sequence conservation during evolutionary courses

Orthologous protein sequences for TTC7A were extracted from National Center for Biotechnology Information (NCBI), which were aligned using ClustalX2 and displayed using BoxShade. TreeView was used to display the phylogenetic distance of the homologous sequences generated through sequence alignment by ClustalX2.

Reverse transcription PCR (RT-PCR)

About 3 ml of ethylenediaminetetraacetic acid (EDTA) whole blood was collected from the patient and her parents. White blood cells (WBC) were isolated by a red blood cell lysis solution (Qiagen Inc., Valencia, CA 91355, USA). Total RNA was extracted from WBC using TRIZOL® Reagent (Invitrogen, Carlsbad, CA 92008 USA). Reverse transcription reaction from 1 mg of total RNA was performed using SuperScript™ II Reverse Transcription reagent (Invitrogen, Carlsbad, CA 92008 USA). PCR using 0.5 μl first-strand cDNA as template was performed using HotStarTaq® plus DNA polymerase (Qiagen Inc., Valencia, CA 91355, USA) system with primers corresponding to sequence from exon 17 (forward primer): TTC7A-cDNA-Exon17F (5′-CCATGAAGAAGCAGAGTGGC-3′) and exon 19 (reverse primer) TTC7A-cDNA-Exon19R (5′-CAT GATGCCACGCCCACCTCTG-3′).

Results

More than 120 million 90-bp paired-end sequencing reads were generated for each sample, reaching >95-fold mean coverage depth for the targeted regions after quality control of the sequencing reads. 92.6% of the targeted sequences were covered at 10-fold or above for all three individual samples. After filtering out the common variants using public and internal databases described above and evaluation of functional impact, a total of 802 variants (including single nucleotide variants [SNVs] and indels) from coding regions (missense, nonsense, or other loss of function mutations [LOF]) and splicing junctions were identified for the patient. Of these variants, one is a de novo mutation, five were homozygous mutations, and six variants in three genes were compound heterozygous mutations. The list of these mutations is presented in Table S1.

Two mutations were detected in TTC7A. These mutations located on paternal and maternal alleles, respectively, and were considered as the causal mutations for the disease based on the recent reports linking mutations in this gene with MIA-CID (8, 9, 13, 14). The
parents were confirmed to be respective carriers of the two mutations through WES and the mutations were also confirmed by Sanger sequencing (Fig. S2). One of the mutations, RefSeq NM_020458.3:exon20:c.G2569T, was inherited from the mother and causes a nonsense mutation that would delete the last two amino acids of the protein (p.E857X). Although the last two amino acids are not located in a tetratricopeptide repeat (TPR), the C-terminus region is highly conserved among orthologs spanning from fishes to mammals and in TTC7B, the closest paralog of TTC7A. This is especially true of the very last amino acid of the protein, leu882 (Fig. S3). Examining the WES data confirmed good coverage of the TTC7A gene on all the 20 exons and no other uncommon exonic or splicing variant (<3%) was detected in either the patient or her parents in this gene.

The mutation inherited from the father, RefSeq NM_020458.3:exon18:c.2018-2A>G, is a variant located in the invariable splice acceptor of intron 17, which is predicted to cause skipping of exon 18 during splicing and would result in an inframe deletion of 45 amino acids. Using a pair of primers located from exons 17 and 19, we amplified a lower band by RT-PCR from the patient and her father, together with a band of normal size (Fig. S4). DNA from the mother and the two normal controls only showed amplification of the band of normal size. The intensity of the lower bands seems to be comparable to the bands of normal size, indicating comparable expression level from the mutant allele for the patient and her father. This is consistent with the inframe nature of the deletion predicted by skipping of exon 18 because no premature stop codon would be introduced by this exon-skipping that would induce nonsense-mediated mRNA decay. The stop codon inherited from the mother occurs at the last exon of the gene, thus is not expected to cause nonsense-mediated decay or a change in the size of the mRNA.

**Discussion**

From a series of studies on the role of TTC7A in MIA-SCID, it was found that mutations in this gene likely explain a large majority of MIA-SCID cases if not all of them (8, 9, 13). The gene has been shown to be one of a list of genes associated with VEOIBD as well (14), likely by hypomorphic mutations (15). Variable phenotypes were observed in reported cases, notably on the presence and absence of lymphocytopenia, and in two of the families reported by Avitzur et al. the absence of apparent signs of intestinal atresia. For all the cases in the three families reported by Avitzur et al. apparent apoptotic enterocolitis (AE) were observed almost immediately after birth (14). While we were preparing this report, an additional case of MIA-CID caused by TTC7A mutations was described (16). Seven of the 26 cases caused by TTC7A mutations survived to various ages and were alive at the time of report. Comparing the genotypes of these cases may reveal a correlation between genetic mutations and the clinical manifestations and prognosis, shedding light on future management of patients. We summarize these cases in Table 2 and Fig. 2 in order to gain a better understanding of the genotype–phenotype correlations for all reported cases of patients with TTC7A mutations in literature.

Most of the causal mutation alleles in this gene were nonsense, frameshift indels, or splicing abnormalities causing the skipping of one or two entire exons. Certain detection bias was likely, as reflected from the study of Avitzur et al. which selected patients not by MIA but by VEOIBD and found TTC7A mutations in 3 of the more than 40 patients examined. This is understandable because IBD is a multigenic complex disease, with enrichment of monogenic causes only when the onset age approaches 6 years or younger, particularly infants (15). For the three remaining studies, TTC7A mutations seem to explain all the genetic causes (8, 9, 13). It is reasonable to predict that more and more missense mutations in this gene of a hypomorphic nature will be detected, which may be associated with various, less severe phenotypes involving the gastrointestinal tract or the immune system.

Despite phenotypic variations among patients with the same genetic mutations (e.g. between C_F2 and C_F3 and the two siblings in A_F3), it appears that there exists a strong correlation between the nature and the position of the mutation and the survival of patients and their phenotypes. As can be seen from Table 2 and Fig. 2, out of the 52 causal mutation alleles from the 26 families, only 10 were missense mutations. Five of the eight patients who carry at least one missense mutation survived to the time of their respective reports. The surviving patients also are likely to have missense mutations not located on regions corresponding to the nine TPRs of the protein or nonsense mutations located in the last exon of the gene, exon 20. When a premature stop codon occurred in the last exon, nonsense-mediated mRNA decay did not occur and normal amount of mRNA was likely to be expressed. In this case, it is possible that residue function of the protein might be retained, which may correspond to a relatively better chance of survival. Interestingly, although the patient from family 1 reported by Avitzur et al. (14) (A_F1) did not survive, she did not have atresia, unlike most other patients with TTC7A mutations. This might be related to the missense mutation she carried, and is not located on a tetratricopeptide repeat (Fig. 2). It is acknowledged that many factors would affect mortality and even for VEOIBD without detectable intestinal atresia, mortality can still be high as in the cases reported by Avitzur et al. (14).

In addition to Family 3 described in Avitzur et al. (A_F3), families described in Samuels et al. (S_F1-6, homozygous Exon7:c.1001 + 3ΔAGAT) and Family 1 described by Chen et al. (C_F1, homozygous Exon16:c.1919 + 1G>A) showed either normal lymphocyte count or mild lymphopenia. Interestingly, both in the cases of S_F1-6 and C_F1, the causal mutations all involve homozygous exonic deletions. Although we do not know for certain the status of the immune system of all these patients, one intriguing possibility is that trace amount of mRNA with normal splicing may exist.
Table 2. Summary of reported mutations on TTC7A

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<th>Report</th>
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<th>Mutations and their functional impact</th>
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<tr>
<td>Samuels et al. (8)</td>
<td>S_F1-6</td>
<td>MIA</td>
<td>Exon7:c.1001+3ΔAAGT→skipping of exon 7</td>
<td>Homozygous</td>
<td>French-Canadian</td>
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<td></td>
<td>S_F7</td>
<td>MIA-CID</td>
<td>Exon7:c.1001+3ΔAAGT→skipping of exon 7</td>
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<td>Chen et al. (13)</td>
<td>C_F1</td>
<td>MIA-CID</td>
<td>Exon16:c.1919+1G&gt;A→skipping of exon 16</td>
<td>Homozygous, consanguineous</td>
<td>Arabic</td>
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<tr>
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<td>C_F2</td>
<td>MIA-CID</td>
<td>Exon2:c.313ΔTATC→p.Y105fs</td>
<td>Heterozygous/consanguineous?</td>
<td>Serbian</td>
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<tr>
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<td>C_F3a</td>
<td>MIA-CID</td>
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<td>C_F4a</td>
<td>MIA-CID</td>
<td>Exon5:c.762ΔG→p.K254fs</td>
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<td>?</td>
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<td>Exon7:c.1001+3ΔAAGT→skipping of exon 7</td>
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<td>French-Canadian and Mixed European</td>
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<td>C_F7a</td>
<td>MIA-CID</td>
<td>Exon9:c.T1196C→p.L399P</td>
<td>Homozygous, nonconsanguine</td>
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<td>C_F8</td>
<td>MIA-CID</td>
<td>Unknown mutations→Exon 2–3 skipping</td>
<td>Heterozygous</td>
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<tr>
<td>Bigorgne et al. (9)</td>
<td>B_FA</td>
<td>MIA-CID</td>
<td>Unknown mutations→Exon 2 skipping</td>
<td>Heterozygous</td>
<td>Mixed European</td>
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<tr>
<td></td>
<td>B_FCa</td>
<td>MIA, CID</td>
<td>Exon20:c.T2468C→p.L823P</td>
<td>Homozygous/likely consanguine</td>
<td>Sri Lanka</td>
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<td></td>
<td>B_FDa</td>
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<td>Exon11-4 bp deletion→Skipping of exon 12</td>
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<td>Intron12:c.1510+105T&gt;G→Skipping of exon 12</td>
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<td>Our study</td>
<td>Y_F1d</td>
<td>MIA-CID</td>
<td>Exon18:c.2018-2A→G→Skipping of exon 18</td>
<td>Heterozygous/ non-consanguine</td>
<td>Malay</td>
</tr>
</tbody>
</table>

AE, apoprotic enterocolitis.

aAlive at the time of writing.
bp.K606R and p.S672P, being located on the same haplotype, were both listed as causal mutations.

cThere are two affected siblings in this family and one of the two was alive at the time of writing.
dPassed away during preparation of this report, at an age of 27 month.
for these patients, enough for the development of thymus but inadequate to relieve gastrointestinal abnormalities. A concrete conclusion on the genotype–phenotype correlation, however, awaits many more reports on the mutations of this gene in human diseases and in-depth functional characterizations. Interestingly, VEOIBD is also commonly observed in SCID patients who have atypical manifestations, caused often by hypomorphic mutations in SCID genes (15), suggesting correlation in the development of the two systems.

There are three spontaneously arising mutations in the mouse TTC7A ortholog, Tic7 (17–19), but little phenotypic similarities were observed between the mouse models and human patients. The flaky skin (fsn) mice showed abnormalities in the immune system such as enlarged peripheral lymph nodes, hyperreactive lymphoid cells, which are quite different from the SCID-like phenotypes observed in human patients. The fsn, int and hea mice all showed papulosquamous skin disorders, and anemia with hematopoietic abnormalities (17–19), also very different from the phenotypes shown in human mutations. Although few gastrointestinal phenotypes were observed in these mice, fsn mice did show severe weight loss with diarrhea and intestinal apoptosis, (14, 20), similar to those seen in the infantile patients reported in Avitzur et al. The difference between manifestations in mouse and human might be explained by potential differences in function between the TTC7A orthologs. Indeed, comparison of these orthologs and their paralogs (TTC7B) showed much more sequence divergence in TTC7A than in TTC7B (Fig. S5), suggesting that TTC7A may have developed newer functions during the course of evolution and significant functional differences exist among its orthologs.

Founder effect was suggested by these studies, most notably in French-Canadians for whom a 4 bp deletion causing exon 7 skipping was identified as the causal mutation for MIA in all six families with French-Canadian ancestry from both parents (8). The same deletion was also found in C_F5 reported in Chen et al. who also has French-Canadian ancestry (13). Exon20:c.T2468C, a mutation that causes p.L823P was found in family 7 in Samuels et al. (S_F7 in Table 2 and Fig. 2) and Family 4 in Chen et al. (C_F4 in Table 2 and Fig. 2), suggestive of a founder mutation in the English ancestry. Homozygous mutations of likely founder effect were also found in a Saudi Arabia family and a Sri Lankan family, respectively (9). Likewise, homozygous deletions (Exon2:c.313ΔTATC) were found in a Serbian family and a Bosniak family, respectively, suggesting the possibility of a Slavian origin for this mutation. Also interesting is that some of the very rare variants detected in public sequencing projects showed absolute conservation during the course of evolution, pointing to the possibility of detrimental alleles existing in the general population (Fig. 3c). This conservation is similar to the reported disease causal mutations (Fig. 3a) and very different from those with high population frequency in the general population (Fig. 3b).
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(a) Disease causal mutations

(b) Common variants in public databases

(c) Rare variants in public databases

Fig. 3. Amino acid sequence conservation for variants in TTC7A. (a) Two of the representative causal mutations reported to cause VEOIBD. (b) Two variants in the gene reported in public databases with relatively common population allele frequency. p.V538L corresponds to rs2304290 in dbSNP with a 27.4% allele frequency from the 1000 Genomes project. p.Q377E corresponds to rs117304542 and has an allele frequency of 0.7% in 1000 Genomes project and 2.5% in CHB + JPT in pilot_1_CHB + JPT_low_coverage_panel. These variants are much less conserved during evolutionary courses. (c) Two rare variants reported for TTC7A in public databases and carried by apparently healthy individuals. p.D511G corresponds to rs139416474 and it was found with one allele in 1000 Genomes and very low estimated population allele frequency; p.Y300C corresponds to rs146719089 and it was found to have 0.0005% allele frequency in EVS ESP6500 dataset. These variants are well conserved during evolutionary courses.

To the best of our knowledge, this is the first report of this syndrome in a Malay as well as in East Asian populations. The information obtained is vital for genetic counseling and prenatal diagnosis for the affected family. Owing to the low awareness of this condition, it is likely many similar cases are undiagnosed. There is a need for larger multi-centre studies on this condition to identify population carrier risk and phenotype–genotype correlations. All these studies may also have implications for population screening of recessive mutations in the future.

Supporting Information
Additional supporting information may be found in the online version of this article at the publisher’s web-site.

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References
6. Ali YA, Rahman S, Bhat V, Al Thani S, Ismail A, Bassioon Y. Hereditary multiple intestinal atresia (HMIA) with severe combined


