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ORIGINAL ARTICLE

Multiple endocrine neoplasia type 1 (MEN1): An update of 208 new germline variants reported in the last nine years

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This review will focus on the germline *MEN1* mutations that have been reported in patients with MEN1 and other hereditary endocrine disorders from 2007 to September 2015. A comprehensive review regarding the analysis of 1336 *MEN1* mutations reported in the first decade following the gene's identification was performed by Lemos and Thakker in 2008. No other similar papers are available in literature apart from these data. We also checked for the list of Locus-Specific DataBases (LSDBs) and we found five *MEN1* free-online mutational databases.

151 articles from the NCBI PubMed literature database were read and evaluated and a total of 75 *MEN1* variants were found. On the contrary, 67, 22 and 44 novel *MEN1* variants were obtained from ClinVar, MEN1 at Café Variome and HGMD (The Human Gene Mutation Database) databases respectively.

A final careful analysis of *MEN1* mutations affecting the coding region was performed.

Keywords MEN1, cancer, mutations, molecular diagnosis, variants

Introduction

MEN1 (Multiple Endocrine Neoplasia Type 1) syndrome is a dominantly inherited disease showing a high degree of penetrance that is characterized by the combined occurrence of tumors in endocrine organs including the pituitary gland, parathyroid gland and pancreatic islets. Some patients may also develop adrenal cortical tumors, carcinoid tumors, facial angiofibromas, collagenomas and lipomas (1,2). The disorder is inherited with an equal sex distribution and the age of onset ranges from 5 to 81 years (3–7). The clinical picture for MEN1 syndrome is largely dependent on the affected glands and on the type of hormonal hypersecretion. All involved tissues can result as affected in relation to the age of the onset of the disease. Parathyroid tumors, which lead to hypercalcemia, are the most common feature of MEN1, since they occur in about 95% of patients (3–7). Pancreatic islet cell tumors, which consist of gastrinomas, insulinomas, pancreatic polypeptidomas (PPomas), glucagonomas and vasoactive intestinal polypeptidomas (VIPomas), occur in about 40% of patients and represent the second most frequently expressed clinical manifestation of MEN1 (8). Anterior pituitary tumors, including prolactinomas, somatotrophinomas, corticotrophinomas or non-functioning adenomas, occur in about 30% of patients (8).

The majority of patients with the inherited form of disease carry germline mutations in *MEN1*, a tumor-suppressor gene located on chromosome 11q13 (9,10). The *MEN1* gene, identified in 1997 by Chandrasekharappa et al. (11), consists of 10 exons and encodes a 610 amino acids protein (MENIN). The 1.83 kb coding region is organized into nine exons and eight introns. The sizes of exons range from 41 to 1.297 bps, while those of introns from 80 to 1.564 bps. Exon 1, the 5′ region of exon 2, and the 3′ region of exon 10 are untranslated. The 1400 bp region upstream of exon 2 displays a strong promoter activity, in particular minimal promoter region is contained between nt −135 and nt −36 (12).

The MENIN protein is conserved from *Drosophila* to humans, but is not present in yeast or *Caenorhabditis elegans* (13). In humans, a single transcript of 2.8 kb (MEN1-001 ENST00000312049.9) that encodes MENIN is detected in most tissues (12). However, six alternative transcripts, not affecting the coding region, plus one very rare variant, resulting in an elongation of the reading frame by 15 bases at the exon 2-intron 2 junction, have been reported (14,15). MENIN is a nuclear protein ubiquitously expressed, but its expression levels

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vary from tissue to tissue. A little amount of MENIN is also detectable in the cytoplasm even in the cell membrane (16). Its nuclear localization sequences (NLSs), located in its C-terminal region, can directly interact with DNA in a sequence-independent manner. Recent progress and knowledge of MENIN suggests that it may act as a scaffold protein that controls gene expression and cell signaling (16).

To date, many germline or somatic mutations have been reported in both MEN1 families and sporadic cases (17–22). The first emerging data are that *MEN1* mutations are distributed through the entire coding region without any significant hot spot region. Approximately 20% of mutations are nonsense mutations, about 50% are frameshift insertions and deletions, 20% are missense mutations while about 7% are splice site defects (8). Furthermore, more than 10% of the *MEN1* mutations arise *de novo* and may be transmitted to subsequent generations (8). Nevertheless, about 10–20% of MEN1 patients may not harbor mutations within the coding region of the *MEN1*: these individuals may, indeed, carry mutations in the promoter or in untranslated regions, although definitive data regarding the mutational status of these two regions are still lacking (8). Because of the growing number of preventive care options available to MEN1 patients and families, the early clinical and genetic identification of "at-risk" individuals is becoming increasingly important. DNA-testing, introduced in 1997, reduces the morbidity and mortality of MEN1 patients (8), allowing clinicians to administer treatment in the early stages of the disease. Genetic testing is therefore recommended for patients who meet clinical criteria for MEN1 and for those to whom a MEN1 diagnosis is suspected. In addition, identification of a specific mutation in a patient allows early detection of healthy carriers and provides an indication for periodic screening for MEN1 (8).

MEN1 mutations are also involved in the etiology of familial isolated hyperparathyroidism (FIHP) which is an isolated endocrinopathy affecting parathyroid glands. It is a hereditary autosomal dominant condition with an age of onset between 20 and 25 years, presenting with symptoms such as hypercalciuria, polyuria, polydipsia, constipation, nephrolithiasis, osteoporosis, neuromuscular changes, lethargy and cognitive disfunction. FIHP can also occur in asymptomatic individuals diagnosed during investigation for another disease. No gene has yet been associated exclusively with FIHP (23). *MEN1* mutations have been reported in 42 families with FIHP (17). A great number of FIHP related *MEN1* mutations (38%) cause an amino acid change in MENIN protein (17).

Since the *MEN1* gene was discovered, only a review including 459 different germline *MEN1* mutations was published by Lemos and Thakker in 2008 (17). No other similar papers are available in literature. For this reason, in this review, we decided to investigate all novel *MEN1* germline variants reported from 2007 (not included in Lemos and Thakker's database (17)) to September 2015.

Methods

We searched the NCBI PubMed literature database for articles in English, published from January 2007 to September 2015, using the keywords "*MEN1* AND *new* AND *mutation*" and "*MEN1* AND *novel* AND *mutation*". We also investigated free-online *MEN1* mutation databases checking for the list of Locus-Specific DataBases (LSDBs) at the web-site of the Human Genome Variation Society (http://www.hgvs.org/dblist/glsdb.html), GEN2PHEN (http://www.gen2phen.org/data/lsdbs) and WAVE (http://bioinformatics.ua.pt/WAVe/).

In addition, the analysis of the number of different mutations involving the same *MEN1* codon was performed: a lollipop plot was built using MutationMapper software (24,25).

Results

Variants research on NCBI PubMed literature database

We obtained 70 and 81 articles using the respective keywords "*MEN1* AND *new* AND *mutation*" and "*MEN1* AND *novel* AND *mutation*". A total of 151 articles were also evaluated. However, most of these papers did not report new *MEN1* variants, so they were rejected with only 36 being selected (references listed in Table S1). Two of these articles (26,27) also contained some variants (p.E26X (26) and p.F447L (27)) that were mis-interpreted from the authors as novel, but these were already present in Lemos and Thakker's database (17).

75 *MEN1* novel variants (Table S1) (Figure 1) were selected during our research on PubMed database: 63 (84%) were associated to MEN1 syndrome, 5 were found in association to sporadic insulinoma (7%), 3 (4%) were associated to FIHP (one missense mutation p.P277L (28) and two splice-site mutations c.824G>T (29) and c.1186-1G>A (27)) and 2 (2.5%) were found in primary hyperparathyroidism with hyperplasia (HPT) (one nonsense variant p.G271X (27) and one missense p.F364C (27)). The remaining 2 (2.5%) (one missense p.P72H and one intronic IVS3+18C>T) were classified as Variant of Uncertain Significance (VUS) (30,31).

Databases investigation

Five *MEN1* publicly available databases were found: (a) Cardiff University's Human Gene Mutation Database (HGMD) (18), (b) Universal Mutation Database (UMD) (19), (c) Leiden Open Variation Database (LOVD) (20), (d) MEN1 at Café Variome (21) and (e) ClinVar (22).

The last update of HGMD and UMD databases has been performed in 2011 and in 2006, respectively. Within HGMD database we found 44 *MEN1* novel variants, 41 of these were MEN1 associated while 3 were found in FIHP patients (Table S1) (Figure 1).

The *MEN1* LOVD database is currently lacking an active curator, while the MEN1 at Café Variome database contains 49 variants, 22 of which were included in Table S1 (Figure 1) as novel. Unfortunately, MEN1 at Café Variome database lacks important information such as the year of variants' annotation and the associated phenotype.

ClinVar database on PubMed seems to be the most updated resource (February 2015). It holds 151 variants, 67 of which were included in Table S1 (Figure 1). 27 out of these 67 ClinVar variants were not classified, while 25 were classified as VUS, 13 were MEN1 associated and 2 were reported as benign variants (p.H199H and p.L83L) (Table S1) (Figure 1).

Figure 2 shows variants' distribution within the examined databases.

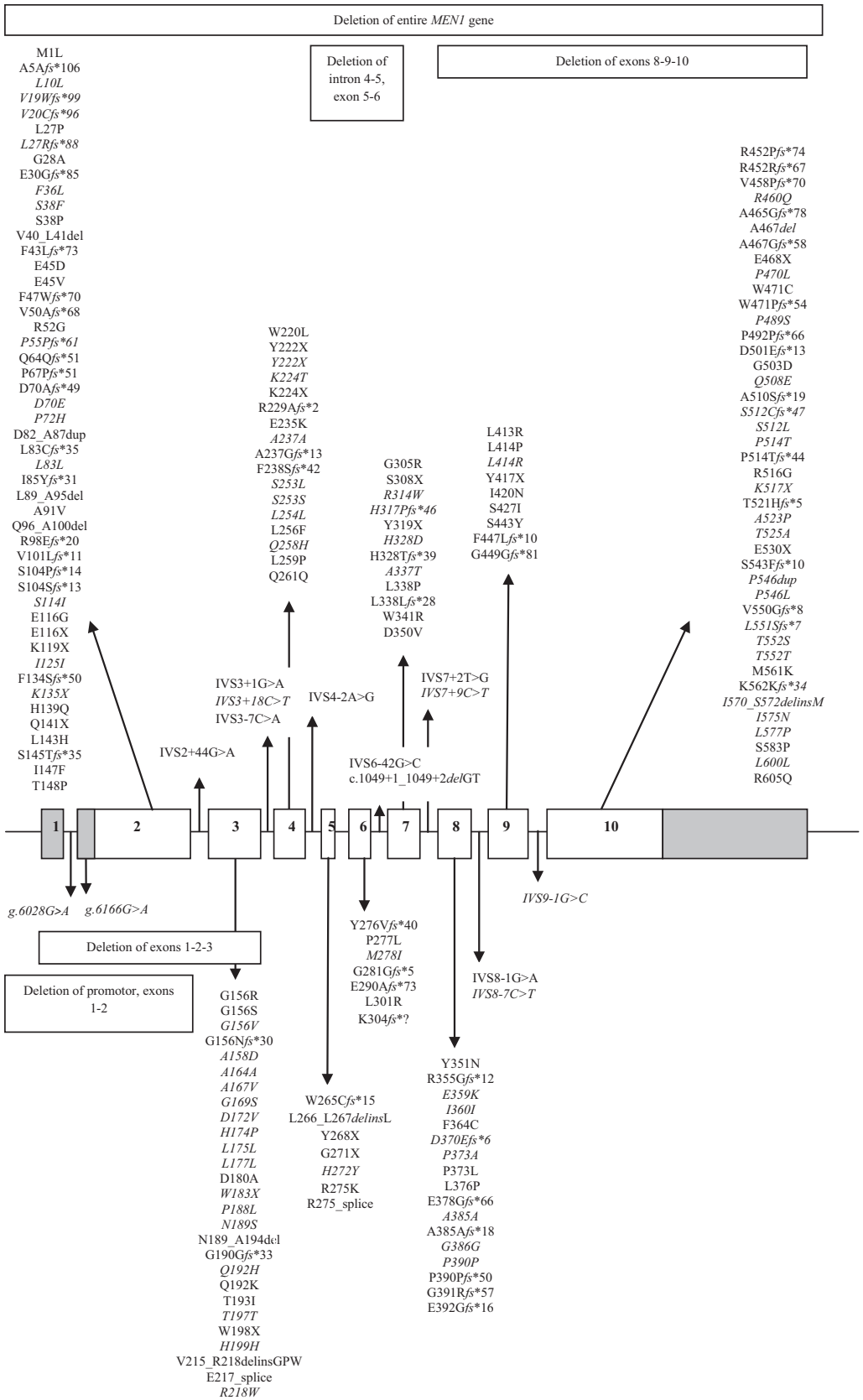

Figure 1 Distribution of 208 novel germline variants on *MEN1* gene reported from January 2007 to September 2015. Rectangles represent *MEN1* exons numbered from 1 to 10, non-coding regions are gray. Mutations are numbered in relation to the *MEN1* DNA reference sequence (GenBank accession number NG_008929.1) or are referred to NP_570711.1. VUS, variants of benign significance and not classified variants are written in italics.

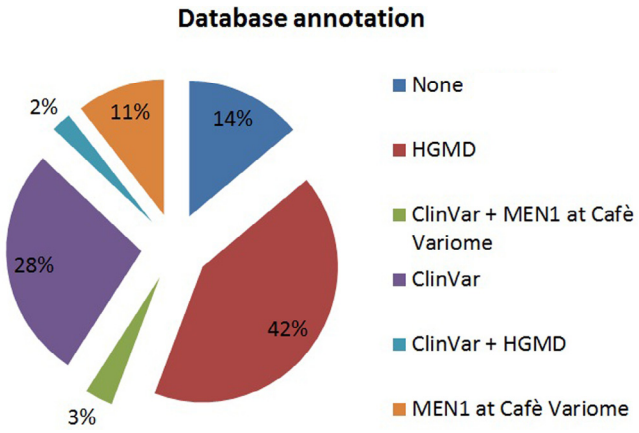

Figure 2 *MEN1* variants distribution within the five databases. The figure shows the distribution of 208 novel germline variants on *MEN1* gene, reported from January 2007 to September 2015, within the five databases: (a) Cardiff University's Human Gene Mutation Database (HGMD), (b) Universal Mutation Database (UMD), (c) Leiden Open Variation Database (LOVD), (d) MEN1 at Café Variome and (e) ClinVar.

Mutation analysis

Figure 3 shows the distribution of 613 *MEN1* mutations affecting its coding region. The most mutated exons resulted to be the 2, 9 and 10, respectively. In particular, within exons 2 and 10, the most common type of mutation was represented by frameshift mutations (Table S2). Codons 139, in exon 2, and 418, in exon 9, were affected by the highest number of mutations (seven different mutations, Figure 3 and Table S2). Finally, codon 516 seemed to be the most mutated codon in exon 10 since it carried five different mutations (Figure 3) (Table S2).

Discussion

The aim of our review was to investigate all novel *MEN1* mutations reported from 2007 to September 2015.

We selected a total of 208 new germline *MEN1* variants (75 from PubMed literature database, 67 from ClinVar database, 44 from HGMD and 22 from MEN1 at Café Variome database) (Table S1) (Figure 1). 117 (56%) of these were associated to MEN1 syndrome, 5 to sporadic insulinoma (2.5%), 6 to FIHP (2.5%), 3 to HPT (1.5%) and 2 were reported as benign variants (1%) (Table S1) (Figure 1). Unfortunately, investigated databases did not provide supporting data for the clinical classification of 76 variants (36.5%). The inability to determine whether these are disease causing or merely represent rare variants not associated with cancer risk generates significant problems in risk evaluation, counseling, and adoption of prophylactic measures.

Considering 576 MEN1 related mutations annotated from 1997 to date, we can assess that frameshift mutations represent the higher rate (42%) while missense mutations show a frequency of 25.5%, nonsense mutations 14%, splice-site mutations 10.5%, in-frame del/ins 5.5% and gross deletions the remaining 2.5%. These results are consistent with previously reported data (8,17) where a more likely association between frameshift deleterious mutations and MEN1 had been described. Most of frameshift mutations, as well as nonsense mutations, are predicted to result in a truncated protein, with the consequent loss of functional domains including the NLSs located in the C-terminal segment. The main effects reported for *MEN1* missense mutations are, indeed, the following: (a) inactivation of MENIN by affecting the function of critical amino acid residues involved in protein interactions and in the tumor suppressor activity; (b) alteration of the capacity of MENIN to regulate the target promoters; (c) reduction of protein stability; (d) enhanced proteolytic degradation (16,17). Most *MEN1* amino acids substitutions reported in the last nine years in PubMed were described as deleterious mutations because changes involved highly conserved amino acids or tracts of

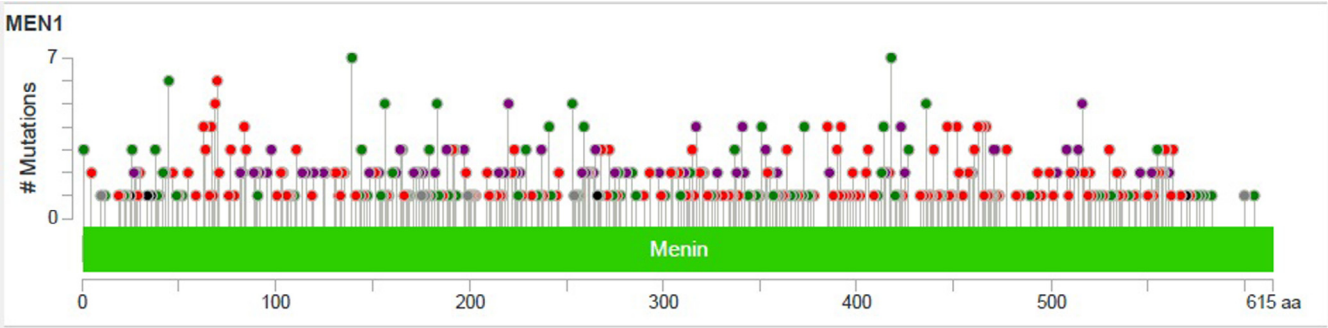

Figure 3 Lollipop plot by MutationMapper reporting all 613 *MEN1* variants (from 1997 to September 2015) affecting coding region. Mutation diagram circles are colored with respect to the corresponding mutation types. Mutation types and corresponding colors are as follows: green for missense mutations, red for truncating mutations (i.e. nonsense, frameshift, splice-site mutations), black for in-frame deletions and insertions, and gray for silent mutations. Circles colored with purple indicate residues that are affected by different mutation types at the same proportion. Codons 139 and 418 result to be affected by the highest number (seven) of mutations (two frameshift deletions and five missense mutations for codon 139 and two in-frame deletions, three missense mutations and two frameshift deletions for codon 418). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

MENIN protein interacting with several targets. A novel substitution p.P72H was reported as a VUS (30). This variant was found in 5 unaffected members of a MEN1 family carrying IVS4+1G>A mutation. Although the authors did not find this variant in 100 alleles from a group of healthy volunteers, they believed that this was a polymorphism (30). However, they stated that future functional studies would have been done. We analyzed p.P72H using SIFT (32) and PROVEAN tools (33). *In silico* analysis identified p.P72H as a neutral variant (0.15 and −1.421, SIFT and PROVEAN score, respectively). These results could strengthen the authors' hypothesis in order to consider p.P72H as a rare polymorphism.

Eight novel in-frame del/ins in MEN1 patients have been discovered in the last nine years (Table S1). Such kind of mutation is more likely to have a deleterious effect on MENIN protein, especially when affecting an interactive domain. In-frame del/ins were analyzed with PROVEAN (33). All obtained scores were compatible with damaging effect on protein (data not shown).

Splice-site mutations are predicted to lead to an accumulation of unspliced precursor mRNA, retention of incompletely spliced precursors or complete absence of transcripts. Generally, a translation of an abnormal mRNA transcript results in truncated and hence inactivated forms of MENIN (17). Six novel intronic variants were found in MEN1 patients in the last years (Table S1). One of these intronic variants (IVS3+18C>T) has been reported by Zha et al. (31) in association with the c.1546_1547insC mutation in a MEN1 family. The authors assessed that this novel intronic nucleotide substitution could be a polymorphism, but it should be analyzed in more patients and controlled groups in order to evaluate its diagnostic relevance (31).

Gross deletions, usually detected by MLPA (multiplex ligation-dependent probe amplification assay) technique (27,34–36), result to be the most rare kind of *MEN1* mutations. The complete *MEN1* gene deletion was reported by different authors (27,37,38). The other gross deletions described affected the first (exons 1–3) (34,35), the central (exons 5 and 6) (36) and the final (exons 8–10) regions of the gene (39).

Five novel *MEN1* mutations, FIHP associated, were reported in the last nine years: three missense mutations and two splice-site mutations (Table S1).

Considering all 37 *MEN1* mutations, FIHP associated, reported from 1997 to September 2015, we can assess that, differently from MEN1 syndrome, missense mutations are more frequent (38%).

Finally, three and five novel *MEN1* mutations were reported in patients with HPT and sporadic insulinoma, respectively (Table S1).

In Lemos and Thakker's analysis, it was pointed out that several *MEN1* germline mutations (c.249_252delGTCT exon 2 codons 83 and 84; c.292C>T exon 2 codon 98; c.358_360delAAG exon 2 codon 120; c.628_631delACAG exon 3 codons 210 and 211; c.784-9G>A intron 4; c.1243C>T exon 9 codon 415; c.1378C>T exon 10 codon 460; c.1546delC exon 10 codon 516; c.1546_1547insC exon 10 codon 516) were found to recur in apparently unrelated affected kindreds, indicating these as potential mutational hot spots (17). On the contrary, considering all *MEN1* germline mutations reported from 1997 to September 2015 worldwide, we evaluated *MEN1* codons most affected by mutations (Figure 3) (Table S2). Most of these codons were located in exons 2, 9 and 10 where deletional and insertional hot spots may be associated with DNA sequence repeats, consisting of long tracts of either single nucleotides or shorter elements ranging from dinucleotides to octanucleotides (40). In particular, deletions and insertions at codon 516 involve a poly$(C)_7$ tract, where a slipped-strand mispairing model is the most likely mechanism to be associated with these mutational events (17,40).

Unfortunately, a correlation between *MEN1* mutations and clinical manifestations of the disorder appears to be absent: in fact the same mutation or different mutations, involving a same codon, can be associated to a wide range of MEN1-related tumors or to FIHP and HPT, not only within the same family but also in sporadic disease forms.

In conclusion, results of our research show that most of novel *MEN1* variants were found within LSDBs but NCBI PubMed literature database seems to be the most complete source of data regarding variants' clinical classification. In fact, as the aim of genetic testing is to provide a diagnosis that must drive medical management and genetic counseling, consistent and transparent criteria regarding the deposition and interpretation of variants are vital. Unfortunately, evaluated databases did not provide consistent data that could facilitate variants' interpretation and, to date, 76 variants (36.5%) are reported as VUS. We think that intensive efforts should be spent in order to provide an increased accuracy of variants' annotation in LSDBs.

Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.cancergen.2015.12.002.

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