# The role of molecular genetics in the clinical management of sporadic medullary thyroid carcinoma

*A systematic review*

Jonathan Mark Fussey FRCS (ORL)[1, 2], Bijay Vaidya PhD[2, 3], Dae Kim PhD[4], Jonathan Clark FRACS[5,6], Sian Ellard PhD[2], Joel Anthony Smith MD[1, 2]

Affiliations:

1. Department of head and neck surgery, Royal Devon and Exeter Hospital, Exeter UK
2. Institute of Biomedical and Clinical Science, College of Medicine and Health, University of Exeter, Exeter UK
3. Department of Endocrinology, Royal Devon and Exeter Hospital, Exeter UK
4. Department of head and neck surgery, St George's University Hospital, London UK
5. Sydney Head and Neck Cancer Institute, Chris O'Brien Lifehouse, Sydney Australia
6. Central Clinical School, University of Sydney, Sydney, Australia

Corresponding author:

J M Fussey

Department of otolaryngology, Royal Devon and Exeter Hospital, Barrack Road, Exeter UK

jfussey@nhs.net

01392 411611

**Abstract**

*Background*

The significant variation in the clinical behaviour of sporadic medullary thyroid carcinoma (sMTC) causes uncertainty when planning the management of these patients. Several tumour genetic and epigenetic markers have been described, but their clinical usefulness remains unclear. The aim of this review was to evaluate the evidence for the use of molecular genetic and epigenetic profiles in the risk-stratification and management of sMTC.

*Methods*

Medline and Embase databases were searched using the MeSH terms “medullary carcinoma”, “epigenetics”, “molecular genetics”, “microRNAs”; and free text terms “medullary carcinoma”, “sporadic medullary thyroid cancer”, “sporadic medullary thyroid carcinoma”, “RET”, “RAS” and “miR”. Articles containing less than ten subjects, not focussing on sMTC, or not reporting clinical outcomes were excluded. Risk of bias was assessed using a modified version of the Newcastle-Ottawa Scale.

*Results*

Twenty-three studies met the inclusion criteria, and key findings were summarised in themes according to the genetic and epigenetic markers studied. There is good evidence that somatic *RET* mutations predict higher rates of lymph node metastasis and persistent disease, and worse survival. There are also several good quality studies demonstrating associations between certain epigenetic markers such as tumour miR-183 and miR-375 expression and higher rates of lymph node and distant metastasis, and worse survival.

*Conclusions*

There is a growing body of evidence that tumour genetic and epigenetic profiles can be used to risk-stratify patients with sMTC. Further research should focus on the clinical applicability of

these findings by investigating the possibility of tailoring management to an individual's tumour mutation profile.

## 1. Introduction

### 1.1 Rationale

Medullary thyroid carcinoma (MTC) is a malignant tumour of the neural crest derived parafollicular C cells. Due to the recent increase in incidence of papillary thyroid carcinoma, MTC now comprises only 1-2% of all thyroid cancers[1], but accounts for a significant proportion of thyroid cancer morbidity and mortality. The rate of regional and distant metastasis at presentation is up to 35% and 13%, respectively[2], and there has been no trend towards earlier stage at diagnosis or improved overall survival in recent decades[3].

Approximately 20-25% of patients have hereditary MTC (hMTC) either as part of the multiple endocrine neoplasia syndromes (MEN2A and MEN2B) or in isolation as familial medullary thyroid carcinoma (FMTC), however in the majority of cases the disease is sporadic (sMTC)[4]. Although around 7% of those with apparently sporadic MTC do indeed harbour germline mutations[5], this group is a minority and is not included in the definition of sMTC used in this article. sMTC often follows an unpredictable course, with some patients suffering rapidly progressive and ultimately fatal disease, some surviving for many decades with incurable but stable disease, and others with relatively indolent disease amenable to cure following surgery.

The gold standard treatment for patients with biopsy proven, node negative sMTC is total thyroidectomy and central compartment neck dissection (CCND)[1, 6]. The role of prophylactic lateral compartment neck dissection is less clear, with subtle differences in the guidance offered by the American Thyroid Association (ATA) and British Thyroid Association (BTA)[1, 6]. There is also a lack of unanimity on the subject of limited surgery for small tumours, with the ATA suggesting that hemithyroidectomy is sufficient if the post-operative serum

calcitonin is less than 1000pg/ml[1], and the BTA using a tumour diameter of less than 5mm as a cut-off [6]. Other authors have advocated thyroidectomy alone without central compartment neck dissection in patients with tumours smaller than 2cm[7].

These subtle but important differences suggest that there is a lack of good evidence to define the best approach when considering the extent of initial surgery in MTC. In reality, many patients receive multiple operations, either to address the lateral compartment lymph nodes armed with information on central compartment occult nodal metastases and post-operative calcitonin levels, or as a result of a conservative approach with subsequent disease recurrence in cervical lymph nodes.

### 1.2 Objective

The difficulty in predicting the clinical course and prognosis at the time of diagnosis makes management planning particularly challenging in sMTC. Currently the role of somatic genetic and epigenetic profiles in the management of sMTC is unknown. Their use in risk stratification may help to guide treatment and allow improved prognostication, as well as more individualised follow-up. It would allow better planning of the initial operation, for example to include a more aggressive neck dissection in cases with a high risk of cervical lymph node metastasis. Furthermore, it would rationalise inclusion criteria for clinical trials in the current era of personalised medicine and a rapidly increasing selection of novel anti-cancer drugs. The objective of this review is to systematically evaluate the current evidence for value of somatic molecular genetic and epigenetic markers in the risk stratification of patients with sMTC.

## 2. Materials and methods

### 2.1 Study design and eligibility criteria

The search strategy and inclusion criteria were outlined in advance in a protocol and registered with the PROSPERO international prospective register of systematic reviews. The protocol can be accessed at https://www.crd.york.ac.uk/prospero/ (registration number: CRD42019131092). All studies reporting the use of genetic or epigenetic markers in risk stratification and prognostication of patients with sMTC were considered. Articles were excluded if they did not focus primarily on patients with sMTC, or contained less than 10 subjects. Articles in languages other than English were excluded, as were articles not published in the last 25 years. Case reports, correspondence, commentaries and reviews were not eligible for inclusion.

### 2.2 Search strategy

The Cochrane library and PROSPERO international prospective register of systematic reviews were searched using a single search term: "medullary thyroid carcinoma" to identify relevant previous reviews on the topic, however none were identified. A search of Medline and Embase using the Ovid platform was then performed using the MeSH search terms "MEDULLARY CARCINOMA", "EPIGENETICS", "MOLECULAR GENETICS", "MICRORNAS"; and free text terms "MEDULLARY CARCINOMA", "SPORADIC MEDULLARY THYROID CANCER", "SPORADIC MEDULLARY THYROID CARCIMONA", "RET", "RAS" and "miR". Finally, a manual search of bibliographies of included studies was performed. The last search was performed on 3rd April 2019. The search log and Boolean operators used are outlined in Appendix 1.

### 2.3 Study selection and data extraction

Titles and abstracts of identified articles were screened for inclusion independently by two assessors based on the criteria outlined in section 2.1. If relevance was not evident after reading the title and abstract then the full text was studied before making a decision. Any disagreement between assessors was settled by a third assessor if consensus could not be reached. A data capture sheet was used to record study design; number of subjects; specific markers investigated; outcome measures; and key findings from included articles.

### 2.4 Data analysis

Due to the variation in study design and outcome reporting, formal statistical meta-analysis was not possible. Therefore, following article identification, key findings were grouped according to the genetic marker used and presented as a narrative synthesis.

### 2.5 Risk of bias assessment

The studies included in this review were not randomised and were not testing an intervention, so tools for assessing the risk of bias in clinical trials were not applicable. A modification of the Newcastle Ottawa scale (Appendix 2) was therefore used to estimate the risk of bias in included studies.

## 3. Results

### 3.1 Search results and study selection

The initial search outlined in section 2.1 identified 1,036 articles. A manual search of bibliographies yielded a further 12 articles giving a total of 1,048. After screening articles for eligibility, and filtering for articles published in the last 25 years and with human subjects 1,025 articles could be excluded. This left 23 articles meeting the inclusion criteria (Figure 1).

### 3.2 Characteristics of included studies

The twenty-three included studies were published between 1996 and 2018, and apart from one randomised controlled trial[8] and one prospective cohort study[9], all were retrospective in design. They contained a combined total of 1,713 patients. Although all studies focused on the use of genetic and epigenetic markers in the risk stratification or prognostication of sMTC, there were a variety of specific markers used. The majority investigated the clinical role of somatic *RET* mutations[8, 10-20], however four included somatic *RAS* mutations[12, 13, 21, 22] and one focussed on somatic *CDKN2C* mutations[23]. Six studies utilised tumour miRNA expression[9, 21, 24-27], two focussed on tumour methylation levels[28, 29], and one on tumour mTOR expression[30]. The general characteristics of included studies are represented in table 1. The risk of bias in the included studies varied significantly, with modified Newcastle Ottawa scores ranging from 3 to 7 out of 8 (8 being highest quality). Sources of potential bias arose from non-consecutive patient selection and inadequate follow-up duration in most cases, although none were deemed unworthy of inclusion solely based on their risk of bias.

### 3.3 Somatic *RET* mutations

Chance heterozygous mutations in somatic tissue will be retained in the clonal progeny of the cell in which they occur, and can occur either as a cause or effect of cancer. Somatic RET mutations are present in up to 66% of sMTC[17, 20], and there is significant evidence to suggest that they are associated with worse outcomes. This was first demonstrated in 1996[20], and later by Elisei *et al* in their study of 100 patients with 10-year follow-up[18]. The authors found a correlation between somatic *RET* mutations and lymph node metastasis at presentation, lower biochemical cure rate following surgery, and worse long-term survival. Other authors have also demonstrated more advanced tumour stage and worse outcomes in patients with somatic

*RET* mutations[16, 17], and improved specificity when combining the use of somatic *RET* mutations and tumour Ki-67 expression levels[15].

Although there are a large number of described somatic *RET* mutations in sMTC, the commonest is the p.Met918Thr (M918T) mutation, which is also the germline mutation responsible for around 95% of cases of MEN2B[1]. The somatic M918T mutation has been identified in up to 68% of sMTC[31]. It is considered to predict the worst outcome[19], and along with the p.Ala883Phe (A883F) mutation is associated with higher rates of lymph node metastasis, multifocality and persistent disease at last follow-up when compared with patients with other somatic *RE*T mutations or none[16]. Double *RET* mutations have also been associated with worse outcomes[11]. A meta-analysis of 23 studies found that somatic *RET* mutations are associated with a higher rate of lymph node metastasis, distant metastasis, advanced stage at diagnosis, recurrence and mortality[32]. The M918T mutated tumour DNA can also be identified in peripheral blood, and there is some evidence that circulating M918T mutated DNA portends a worse overall survival and more accurately predicts outcome than calcitonin doubling time[10].

Given the established role of RET in hMTC and that almost half of sMTCs harbour somatic *RET* mutations, most efforts at novel drug development have focussed on inhibiting RET and RET-related pathways (Figure 2). This has resulted in the licencing of tyrosine kinase inhibitors (TKI) with specificity for RET, vandetanib and cabozantinib. Both drugs have been found to be superior to placebo in double blind randomised controlled trials in terms of progression free survival in populations consisting of both hMTC and sMTC[8, 33]. In the original vandetanib trial, an M918T *RET* mutation was identified in 142 of 298 patients with sMTC, and subgroup analysis revealed a higher response rate and superior progression-free survival in these patients[8]. However, a recent meta-analysis of studies on vandetanib found minimal evidence of significant benefit, with no evidence of improved overall survival and a significant risk of side effects, which may significantly impact quality of life[34]. It is notable that the studies included in this meta-analysis consisted of patients with both hMTC and

sMTC, and subgroup analysis according to somatic mutational profile was not possible. It is conceivable that stratification by genetic profile may have altered the results given the evidence of improved response rates of M918T RET sMTC.

More recently, two selective RET inhibitors have been developed which are still being evaluated in clinical trials. LOXO-292 is under investigation in a phase 2 study, however preliminary results have demonstrated preclinical activity against various RET alterations. Furthermore, its use in a patient with advanced sMTC with a confirmed somatic RET M918T mutation resulted in tumour regression, a drop in serum calcitonin and symptom improvement[35]. The second drug, BLU-667 is also a selective RET inhibitor still in phase 2 trials, however once again preliminary data has shown a good response in a patient with sMTC and multiple RET mutations[36]. Clearly it is too early to draw conclusions on the efficacy of these novel drugs or the relative effects in patients with different RET mutations, however these preliminary results are promising.

### 3.4 Somatic *RAS* mutations

Somatic *RAS* mutations are present in 30% of all human cancers[37]. In sMTC, somatic *RAS* mutations have been identified in up to 81% of *RE*T negative tumours, and almost never co-exist in *RET* mutated tumours[12, 13, 22, 30]. In contrast to follicular cell thyroid cancers, MTC tends to harbour *HRAS* mutations most commonly and *NRAS* mutations rarely, with the commonest being *HRAS* p.Gln61Arg[38].

The clinical significance of somatic *RAS* mutations has been less thoroughly investigated, and although some data suggests that they predict a more favourable outcome compared to somatic *RET* mutations in sMTC, none of the studies identified in this review were able to demonstrate this with statistical significance[12, 13, 22]. Moura *et al* divided their 2011 cohort of sMTC patients into four groups depending on the presence of high-risk somatic *RET* mutations (M918T and

A883F), other *RET* mutations, *RAS* mutations and no mutations[38]. They found that tumours with somatic *RAS* mutations behaved less aggressively than those with high risk *RET* mutations, but more aggressively than those with other *RET* mutations. Although Cavedon *et al* were unable to identify a significant association between somatic *RAS* mutation and favourable outcome; they did find that tumour miR-224 under-expression correlated with shorter overall survival, and that somatic *RAS* mutations correlated with miR-224 over-expression[21]. They therefore concluded that somatic *RAS* mutations in sMTC predict a less aggressive phenotype.

### 3.5 Other genetic pathways

In up to 45% of sMTC, no somatic *RET* or *RAS* mutation is identified. This has led some authors to investigate the role of other related pathways, as well as epigenetic regulation of gene expression. In general, the quest for novel genes regulating tumorigenesis in MTC has focused on oncogenes in other cancer types, and been relatively fruitless[39]. However, exome sequencing on a patient with MTC and no identifiable *RET* mutation has identified a germline frameshift c.948delT mutation in the *ESR2* gene[40]. This mutation was also present in 3 family members with C-cell hyperplasia, and immunohistochemical studies confirmed its effect being the loss of ERβ and overexpression of RET. This finding of indirect up-regulation of RET expression leading to MTC, provides an interesting insight into alternate pathways for developing MTC, although it has yet to be replicated by other researchers, and its role in sMTC is as yet unknown[41].

The *CDKN* gene family, encoding cyclin-dependent kinase inhibitors has attracted some interest in the setting of MTC. These genes play a vital role in cell cycle control and have been implicated in many cancers, suggesting a tumour suppressor role[42]. Somatic copy number loss in the *CDKN2C* gene has been identified in 19% of a cohort of 62 sMTC cases, and associated with higher rates of distant metastasis and worse overall survival[23]. Genotyping of the *CDKN*

genes revealed a significant association between single nucleotide polymorphisms (SNPs) in the CDKN1B and CDKN2A genes and susceptibility to sMTC[43]. Both of these genes encode cyclin dependent kinases with cell cycle control roles, with the latter being an important stabiliser of the p53 tumour suppressor protein. More recently, fluorescence in situ hybridisation has been used to identify loss of heterozygosity in *CDKN2C* and *CDKN2D* in formalin fixed paraffin embedded MTC samples, raising the possibility of a clinically useful prognostic marker[42].

Based on evidence that the *CDK/RB* pathway may be an alternative target for treatment of MTC, *in vitro* research has demonstrated that dinaciclib (a CDK1/2/5/9 inhibitor) reduced mRNA levels of CD7 and *RET* in MTC cells[44]. The effect was synergistic when dinaciclib was used in combination with a TKI, and the authors postulate the possibility of its use to improve therapeutic *RET* targeting in MTC.

The mTOR pathway is activated in both hMTC and sMTC[45], and there is some evidence that high mTOR activity as measured by tumour p-S6 expression is associated with more invasive sMTC and higher rates of lymph node metastasis[30]. Inhibitors of the mTOR pathway have therefore been investigated in advanced MTC, with a phase II trial of Everolimus showing some benefit[46].

**3.6 Epigenetic markers in sMTC**

Epigenetics can be defined as stably inherited modulations in the expression of genes without altering DNA sequence[47]. The epigenetic control of gene expression is known to influence oncogenesis and tumour behaviour in many cancer types, mainly via aberrations in the histone acetylation and methylation pathways[48]. Although there has been little research on methylation profiles in MTC, a recent study by Ceolin *et al* found that global DNA methylation levels in peripheral blood leucocytes were higher in sMTC patients than hMTC patients[28]. The authors

were unable to identify any correlation between DNA methylation levels and tumour characteristics or clinical outcome.

A significant increase in gene expression of the histone methyltransferases EZH2 and SMYD3 has been found in samples from MTC patients with local and distant metastasis, irrespective of germline and somatic *RET* and *RAS* mutation status[49]. This adds further weight to the suggestion that MTC tumour progression may be controlled by epigenetic factors independent of *RET* and *RA*S pathways. Telomerase activity is controlled by the *TER*T gene, which is usually suppressed in normal tissue, but activated in many human cancers to allow cell proliferation. Although *TERT* gene promoter mutations have not been identified in MTC[50], *TERT* copy number gain has been demonstrated in a subset of patients with sMTC, as well as increased methylation of the *TERT* promoter region in both hMTC and sMTC patients[29]. The same group found a statistically significant association between higher TERT methylation index, and poorer disease-free and overall survival. They postulated that the methylation of the TERT promoter region stimulates TERT expression and telomerase activation thereby contributing to more aggressive disease.

MicroRNAs (miRNAs) are molecules capable of down-regulating gene expression, and have thus been implicated in both carcinogenesis via down-regulation of tumour suppression genes, and tumour progression. Overexpression of miR-183 and miR-375 have been identified in sMTC compared with hMTC, and are associated with increased rates of lateral cervical lymph node metastases, distant metastases and mortality[27]. Other investigators have also identified a positive association between miR-375 levels and tumour stage at diagnosis, lymph node metastasis, calcitonin level at diagnosis and disease progression, independent of *RET* and *RAS* status[25].

miR-183 has been found to down-regulate the expression of the pro-apoptotic gene *PDCD4*[51], and has been implicated in the oncogenesis of other cancers[52, 53]. Down regulation of *PDCD4*

in association with overexpression of miR-29 is a possible carcinogenic factor in both sMTC and hMTC[54]. More recently, the expression of six miRNAs was measured in a cohort of 54 sMTCs, and miR-183 was again found to correlate strongly with lymph node metastasis. In addition to miR-183, miR-21 expression was also found to correlate with lymph node metastasis as well as tumour size, baseline serum calcitonin and T3/T4 tumours at presentation[24]. The authors postulated that routinely testing this marker could guide surgeons in the extent of cervical lymph node dissection. Table 2 summarises the prognostic effects of various somatic genetic and epigenetic changes.

The recent interest in epigenetic factors in sMTC has led some to investigate the feasibility of targeting epigenetic regulators with the aim of developing novel therapeutic agents. As early as 2005, *in vitro* experiments revealed the efficacy of histone deacetylase inhibitors in supressing proliferation and inducing apoptosis in thyroid cancer cell lines[55]. miRNA therapy has now been trialled in other forms of human cancer, with the first molecule to be studied being MRX34, an miR-34a mimic which supresses oncogenes targeted by miR-34a[56]. Initial phase I studies showed promising anti-tumour efficacy, but significant adverse effect profiles. Interestingly, miR-34a expression has been found to be raised in MTC, rather than reduced as in other cancers[57]. Furthermore, Lassalle et al have demonstrated *in vitro* that overexpression of miR-375, one of the most studied miRNAs in MTC, results in improved efficacy of vandetanib on cancer cells[58].

## 4. Discussion

### 4.1 Summary of key findings

This review demonstrates the emerging complexity of the molecular genetic and epigenetic landscape in sMTC. There is now a significant body of evidence supporting the use of various genetic and epigenetic markers in risk stratification of patients with sMTC, however few

authors have used this information to personalise management plans. Currently the clinical stage and serum calcitonin levels dictate the extent of initial and subsequent surgery in these patients, although with increasing availability of genetic profiling of tumours, the incorporation of tumour-specific genetic and epigenetic markers into the decision making process is becoming more feasible.

There is substantial evidence that somatic *RET* mutations predict more aggressive tumour behaviour and a worse outcome, however it is not clear whether these mutations actually drive tumorigenesis in sMTC. Some patients demonstrate heterogeneity of *RET* mutation in cell subpopulations both within different areas of the same primary tumour, and within different metastases from the same patient[7, 59]. This suggests that the mutation is not an early event in tumorigenesis, and incidentally may also explain the wide variation in reported frequency, as its detection depends on which part of a tumour is tested. Furthermore, the presence of somatic *RET* mutations is not unique to MTC and may represent a marker of poor prognosis in many cancers, with various mutations, including M918T, having been identified in breast, lung and liver cancers, correlating with advanced disease stage and poor survival[60-62]. This suggests that the somatic *RET* mutation may play more of a role in tumour progression than tumorigenesis.

The role of somatic *RAS* mutations in predicting clinical course is still not well understood, and the findings of some authors that their presence predicts more favourable outcomes may simply reflect the fact that these tumours almost always lack somatic *RET* mutations. Up to 45% of sMTC express neither *RET* or *RAS* somatic mutations, which suggests a role for other mechanisms. The field of epigenetics is growing rapidly, and there is increasing evidence for a role for several epigenetic markers in risk stratification of sMTC. The most promising of these are miR-21, miR-183 and miR-375, which have been shown by several authors to be associated with increased risk of lymph node metastasis in sMTC[24, 25, 27]. These markers could complement serum calcitonin levels and stage of disease in helping to guide decision-making

when considering the need for lateral compartment neck dissections in the absence of clinical or radiological evidence of nodal metastases.

A significant barrier to the use of these tumour specific markers in risk stratification and management planning is the fact that they are frequently not available at the time of initial diagnosis as their detection relies upon analysis of excised tumour tissue. The work of Cote *et al* on the detection of circulating *RET*-mutated tumour DNA[10], and Romeo *et al* on circulating miR-375[9] is therefore particularly interesting. In addition to their potential use in prognosticating and planning surgical management, genetic and epigenetic characteristics of sMTC tumours may also be valuable in predicting response to novel therapies and developing new therapeutic agents. The improved response rate to vandetanib amongst patients with somatic *RET* M918T mutations demonstrated by Wells *et al,* and the suggestion by Lassalle *et al* that tumour miR-375 overexpression increases susceptibility to vandetanib raise the possibility of using somatic mutation profiles to guide medical therapy[8, 58].

**4.2 Limitations**

The field of molecular genetics in MTC is rapidly evolving and there is substantial evidence in the form of retrospective studies to support associations between various tumour genetic and epigenetic markers and clinico-pathological features. However, the number of studies is still relatively limited, and with each using different molecular genetic characteristics and slightly different outcome measures, comparison of findings is difficult. Furthermore, there is a dearth of prospective evidence on the use of these markers to inform management of patients with sMTC. The majority of research to date has focussed on somatic *RET* mutations, and whilst the evidence for their role in predicting tumour behaviour is strong, it is clear that other molecular genetic mechanisms play a role. Further research is required to confidently base management decisions on these genetic and epigenetic characteristics.

### 4.3 Conclusions

Currently clinical stage and calcitonin levels dictate the extent of initial and subsequent surgery for sMTC, with novel therapies being reserved for local or distant disease progression. The evidence presented in this review suggests that somatic *RET* mutations and tumour overexpression of certain miRNAs may predict aggressive tumour behaviour and lymph node metastasis. There appears therefore to be scope to improve risk stratification in sMTC. This could allow for more individualised decision-making, with the extent of surgery being tailored to patients. Due to the rarity of these cancers, and as analysis of somatic mutations and epigenetic expression is not routinely analysed in most centres, a subspecialist or even nationalised approach to such cases may be warranted. Further research should aim to further clarify molecular genetic and epigenetic drivers of sMTC in order to provide more accurate prognostic information, inform management strategies and provide potential targets for novel therapeutic agents.

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**Table 1: General study characteristics**

| Author | Year | Design | N= | Markers | Main findings |
|---|---|---|---|---|---|
| Ceolin[28] | 2018 | Retrospective | 24 | Global DNA methylation levels | Higher global DNA methylation in sMTC than hMTC, but no correlation with tumour characteristics and clinical outcome |
| Aubert[24] | 2018 | Retrospective | 54 | Tumour miR-183 and miR-21 expression | Both miR-21 and miR-183 were associated with lymph node involvement, and miR-21 was identified as an independent prognostic factor for lymph node involvement. |
| Romeo[9] | 2018 | Prospective | 33 | Circulating miR-375 | Circulating miR-375 levels were able to distinguish between patients with persistent sMTC and those in remission. Distant metastasis was higher and overall survival lower in those with higher levels of circulating miR-375. |
| Cote[10] | 2017 | Retrospective | 75 | Circulating *RET* M918T | Circulating *RET* M918T mutated DNA found in 32% of those with the mutation identified in tissue biopsy, and strongly correlated with worse overall survival |
| Cavedon[21] | 2017 | Retrospective | 107 | Somatic miR-224 and *RAS* | Positive association between miR-224 and *RAS.* miR-224 was a positive prognostic marker, inversely associated with persistent/progressive disease, calcitonin and disease-related death. |
| Galuppini[25] | 2017 | Retrospective | 104 | Tumour miR-375 expression | miR-375 expression was associated with tumour size, capsule invasion, lymph node involvement and stage at diagnosis, although 26 hMTCs were included in the analysis. |
| Romei[11] | 2016 | Retrospective | 70 | Somatic *RET* | Positive correlation between poor prognosis and number of somatic *RET* mutations identified |
| Grubbs[23] | 2016 | Retrospective | 62 | Somatic *CDKN2C* copy number loss | Increased distant metastasis and reduced overall survival in the presence of Somatic *CDKN2C* copy number loss |
| Wang[29] | 2016 | Retrospective | 39 | Somatic *TERT* promoter methylation | Lower overall and disease-specific survival in patients with high *TERT* promoter methylation |
| Simbolo[12] | 2014 | Retrospective | 20 | Somatic *RET* and *RAS* mutations | No significant association between somatic mutation and clinical outcomes. |
| Lyra[30] | 2014 | Retrospective | 77 | Tumour mTOR activation | Increased mTOR activation (as measured by p-S6 expression) associated with lymph node metastasis and invasive tumours, although 10 hMTCs were included in the analysis. |
| Ciampi[13] | 2013 | Retrospective | 175 | Somatic *RET and* RAS mutations | Non-significant correlation between somatic *RAS* mutations and better outcomes. Somatic *RET* mutations predicted worse biochemical cure rates and higher disease progression rates than both *RAS* negative *RET* negative and *RAS* positive *RET* negative cases. |
| Mian[26] | 2012 | Retrospective | 34 | Somatic expression of miR-21, miR-127, miR-154, miR-224, miR-323, miR-370, miR-9*, miR-183, and miR-375 | miR-224 expression levels inversely associated with nodal metastasis and disease stage, and positively associated with biochemical cure. |
| Romei[14] | 2012 | Retrospective | 160 | Somatic *RET* M918T mutations | Somatic *RET* M918T mutations were associated with larger primary tumours. |

| | | | | | |
|---|---|---|---|---|---|
| Wells[8] | 2012 | RCT | 298 | Somatic *RET* M918T | Phase III clinical trial on vandetanib, which found a higher response rate to the drug in sMTC patients with the somatic *RET* M918T mutation. |
| Abraham[27] | 2011 | Retrospective | 12 | Tumour miR-183 and miR-375 expression | Increased expression of miR-183 and miR-375 correlated with lateral compartment nodal metastasis, distant metastasis and mortality, although 7 hMTCs were included in the analysis. |
| Mian[15] | 2011 | Retrospective | 60 | Somatic *RET* and *Ki-67* expression | Both somatic *RET* and tumour Ki-67 expression correlated positively with tumour size, nodal metastasis, distant metastasis and low overall survival. |
| Moura[22] | 2011 | Retrospective | 65 | Somatic *RET* and *RAS* mutations | No statistically significant differences in clinicopathological parameters between *RAS* positive and *RAS* negative cases |
| Moura[16] | 2009 | Retrospective | 51 | Somatic *RET* mutations | Tumours with somatic *RET* mutations affecting exons 15 and 16 were associated with higher rates of lymph node metastasis, redisual disease, advanced disease and persistently raised calcitonin compared with tumours harbouring other *RET* mutations or no *RET* mutation. |
| Dvorakova[17] | 2008 | Retrospective | 48 | Somatic *RET* mutation | Somatic *RET* mutations were associated with more advanced stage at presentation, but not with other clinical and pathological characteristics. |
| Elisei[18] | 2008 | Retrospective | 100 | Somatic *RET* mutation | Somatic *RET* correlated with advanced stage a presentation and worse overall survival (10 years follow-up). M918T somatic *RET* mutations correlated with larger tumours, nodal metastases and distant metastases. |
| Schilling[19] | 2001 | Retrospective | 34 | Somatic *RET* mutation | Somatic M918T *RET* mutations were associated with higher rates of distant metastasis, lower metastasis-free survival and lower overall survival. |
| Romei[20] | 1996 | Retrospective | 18 | Somatic *RET* mutations | Somatic *RET* mutations were associated with higher rates of post-treatment recurrence or high serum calcitonin. |

**Table 2. Summary of prognostic biomarkers in sporadic medullary thyroid carcinoma (WT=wild-type; DMFS = distant metastasis free survival; DFS = disease free survival; OS = overall survival; NS = not statistically significant)**

| Biomarker | Outcome | | | | | | | References |
|---|---|---|---|---|---|---|---|---|
| | Stage at presentation | Calcitonin at presentation | Lymph node metastasis | Distant metastasis | Persistent disease at follow up | DFS | OS | |
| Somatic *RET* M918T or A883F versus *RET* wild-type | | | ↑ | ↑ | ↑ | | ↓ | Moura 2009[16]<br>Schilling 2001[19] |
| Somatic *RET* mutation versus *RET* wild-type | ↑ | | ↑ | | ↑ | | ↓ | Elisei 2008[18]<br>Dvorakova 2008[17]<br>Ciampi 2013[13] |
| Tumour miR-375 over-expression | | ↑ | ↑ | ↑ | | | ↓ | Abraham 2011[27]<br>Galuppini 2017[25] |
| Tumour miR-183 over-expression | | | ↑ | ↑ | | | ↓ | Abraham 2011[27] |
| Tumour miR-21 over-expression | ↑ | ↑ | ↑ | | | | | Aubert 2018[22] |
| Tumour miR-224 under-expression | ↑ | ↑ | | | ↑ | ↑ | | Cavedon 2017[21] |
| Tumour *TERT* hypermethylation | | | | | | ↓ | ↓ | Wang 2016[29] |
| Somatic *CDKN2C* copy number loss | | | | | | | ↓ | Grubbs 2016[23] |
| Somatic *RAS* mutation versus *RAS* wild-type | | | ↓ (NS) | ↓ (NS) | ↓ (NS) | | | Ciampi 2013[13] |

## Figure Legends

Figure 1: Preferred Reporting Items for Systematic reviews and Meta-Analyses 2009 (PRISMA) diagram

Figure 2: Schematic representation of selected downstream signalling pathways activated by *RET*, with sites of possible novel therapy action. Vandetanib and cabozantinib directly inhibit *RET*. Tipifarnib is a farnesyltransferase inhibitor which ultimately inactivates *RAS.* Sorafenib is a tyrosine kinase inhibitor, which deactivates the RAS/RAF/MEK/ERK pathway. Everolimus binds to a protein receptor, which directly inhibits mTOR. (PI3K = phosphoinositide 3-kinase; AKT = protein kinase B; mTOR = mammalian target of rapamycin; RAF = rapidly accelerated fibrosarcoma kinase; MEK = MAPK/ERK kinase; ERK = Extracellular signal kinase)

## Data sharing statement:

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Figure 1

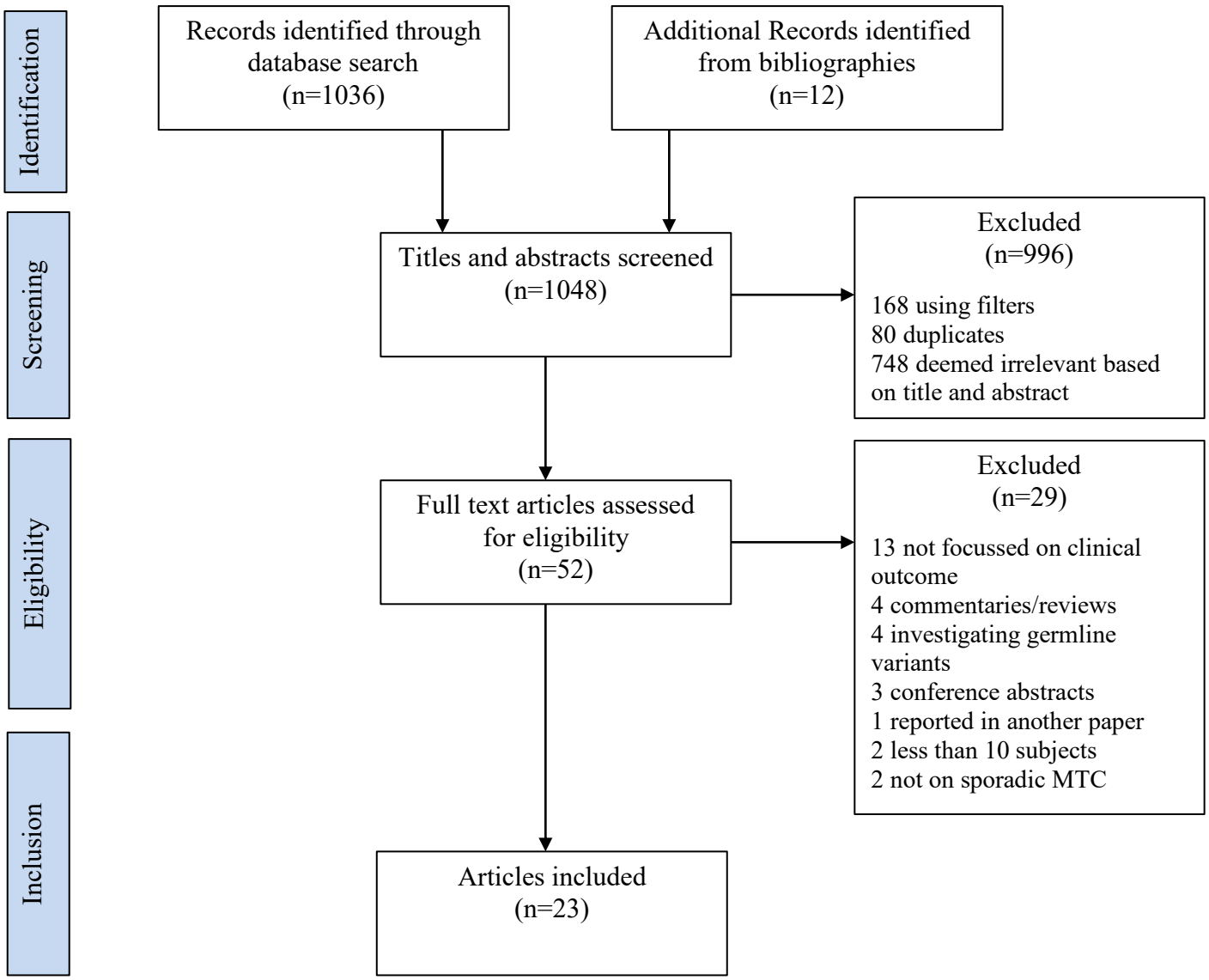
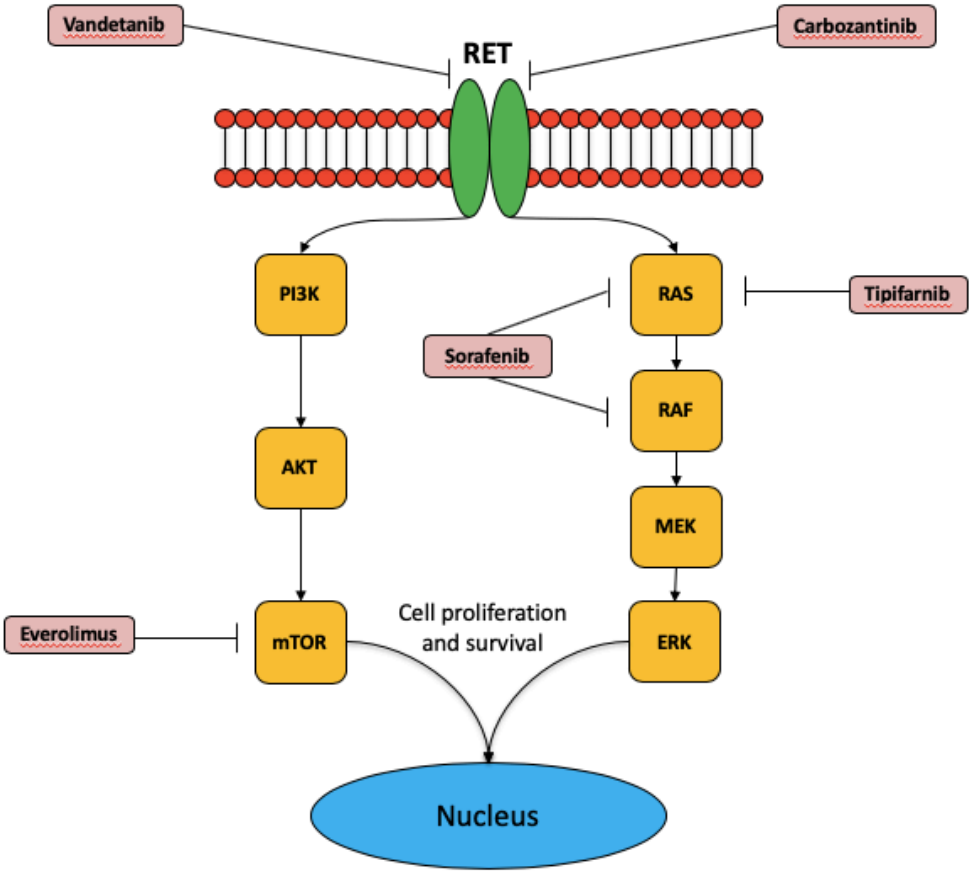



Figure 2




Appendix 1: Search Log

| # | Searches | Results | Type |
|---|---|---|---|
| **1** | Medullary carcinoma.sh | 2473 | Advanced |
| **2** | Medullary carcinoma.af | 13184 | Advanced |
| **3** | Sporadic medullary thyroid cancer.ti | 51 | Advanced |
| **4** | Sporadic medullary thyroid carcinoma.ti | 224 | Advanced |
| **5** | 1 or 2 or 3 or 4 | 13315 | Advanced |
| **6** | Epigenetics.sh | 63624 | Advanced |
| **7** | Molecular genetics.sh | 147654 | Advanced |
| **8** | RET.ti | 5511 | Advanced |
| **9** | RAS.ti | 35313 | Advanced |
| **10** | microRNAs.sh | 64738 | Advanced |
| **11** | miR.ti | 54590 | Advanced |
| **12** | 6 or 7 or 8 or 9 or 10 or 11 | 352177 | Advanced |
| **13** | 5 and 12 | 1036 | Advanced |
| **14** | Limit 13 to English language | 966 | Advanced |
| **15** | Limit 14 to human | 879 | Advanced |
| **16** | Limit 15 to yr= '1994-current' | 868 | Advanced |
| **17** | Limit 16 to humans | 868 | Advanced |
| **18** | Remove duplicates from 17 | 788 | Advanced |
| **19** | From 17 keep 3, 8, 11, 13, 19-20, 63-64… | 34 | Advanced |
| **20** | From 18 keep 3, 8, 10-11, 13, 19-20, 30… | 40 | Advanced |

Appendix 2: Modified Newcastle Ottawa scale for assessing risk of bias

| **1. Patient selection** | **Score** |
|---|---|
| Was case definition adequate? | |
| Was selection consecutive? | |
| Were community controls used? | |
| Were controls defined? | |
| Were controls comparable to cases? | |
| **2. Data collection** | |
| Was outcome assessed by record linkage? | |
| Was follow-up long enough for events to occur? | |
| Were all subjects accounted for in follow-up? | |
| **Total** | |