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Multiple Endocrine Neoplasia Type 2

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MEDULLARY THYROID carcinoma (MTC) is a tumour of the thyroid C-cells. It can occur sporadically or as part of three familial cancer syndromes: multiple endocrine neoplasia type 2A (MEN 2A), MEN 2B and familial MTC (FMTC) [1] (Figure 1). Phaeochromocytoma (PC), a tumour of the adrenal chromaffin cells, parathyroid hyperplasia or adenoma and MTC are observed in MEN 2A (Table 1). Affected family members, however, do not necessarily have all three disease components. While MTC occurs in the majority of affected individuals, only 40–50% of patients with MEN 2A or 2B are diagnosed with PC [2]. Parathyroid disease is detected in 10–35% of MEN 2A cases [2–4]. MEN 2B is distinguished from MEN 2A by the absence of parathyroid disease and the presence of mucosal neuromas, marfanoid habitus and, less frequently, diffuse intestinal ganglioneuromatosis, thickened corneal nerves, skeletal abnormalities and delayed puberty [1, 5]. In FMTC, the only disease feature is MTC with PC and parathyroid disease absent [6].

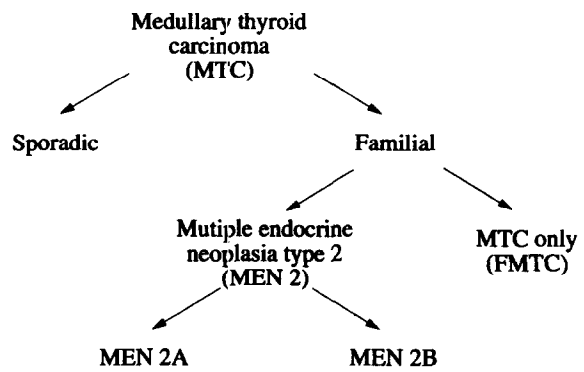

Figure 1. Classification of MTC.

Table 1. Criteria for diagnosis of MTC-associated syndromes [33]

Diagnosis	Criteria
MEN 2A	MTC with either phaeochromocytoma or parathyroid disease or both
MEN 2B	MTC with phaeochromocytoma and associated clinical abnormalities including mucosal neuromas and marfanoid habitus
FMTC	MTC and all living affected and at risk individuals screen negative for phaeochromocytoma or parathyroid disease

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The tissues involved in MEN 2A, 2B and FMTC are linked by a common embryological origin. The C-cells of the thyroid, chromaffin cells of the adrenal and ganglia of the sympathetic plexus of the intestinal tract all originate from the neural crest.

MEN 2A, 2B and FMTC are all inherited as autosomal dominant diseases. An affected family member has a 50% chance of passing the disease on to each of his or her offspring. Thus, when an individual is diagnosed with MTC, it is important to ascertain whether there is a family history of MTC and/or any of the associated features found in MEN 2A or 2B. Biochemical screening tests can be used in presymptomatic identification of MTC or its presumed precursor lesion C-cell hyperplasia, PC or parathyroid disease. Prospective screening for MTC is performed by stimulation of calcitonin release by calcium infusion or pentagastrin injection [7]. An elevated response to stimulation is indicative of the presence of C-cell hyperplasia, MTC or residual recurrent disease in a post-operative individual. Screening for PC includes measurement of blood pressure and quantitation of urinary or plasma catecholamines [4]. Parathyroid disease can be screened for by measuring plasma calcium and parathyroid hormone [4]. A negative response to any of these tests does not imply that the individual has not inherited MEN 2 or FMTC, but only that they do not currently have detectable signs of the disease. Consequently, at risk individuals must undergo repeated screening. The usual treatment offered in response to repeated positive screening tests is surgery.

Recent studies have identified the gene responsible for MEN 2A, 2B and FMTC, providing the opportunity for direct mutation detection in patients with these diseases.

LOCALISATION OF THE *MEN2* GENE

The disease locus for MEN 2A, 2B and FMTC was localised by a combination of genetic and physical mapping techniques. Initially, genetic linkage analyses, using families with MEN 2A, indicated that the disease locus lay in the pericentromeric region of chromosome 10 [8–10]. Subsequent analyses of families with MEN 2B and FMTC confirmed that these disease loci were also located in this chromosome 10 region [11, 12]. With the development of new polymorphic DNA marker loci, including microsatellite and centromeric repeat polymorphisms, it was possible to refine this localisation to a smaller interval on the long arm of the chromosome in 10q11.2 [11]. In 1993, Gardner and associates [13] were able to genetically map MEN 2A to an interval flanked by DNA markers D10S141 and D10S94, using a meiotic mapping panel of recombination events in chromosome 10q. Physical mapping of this region by pulsed field gel electrophoresis and construction of yeast artificial chromosome (YAC) contigs demonstrated that the *MEN 2A* locus lay within a 480-kb interval [14-16]. The *RET* proto-oncogene was mapped

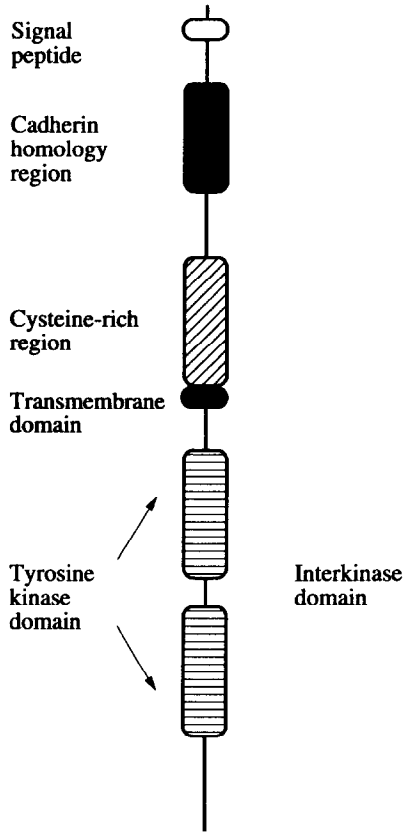

Figure 2. Schematic representation of the ret tyrosine kinase receptor. The functional domains of ret are indicated.

within this defined interval [14, 15] and was thus considered an important candidate for the *MEN 2* gene.

THE *RET* PROTO-ONCOGENE

The *RET* gene encodes a receptor tyrosine kinase with a cytoplasmic tyrosine kinase domain and a large extracellular domain containing a region of cadherin homology [17–19] (Figure 2). In man, the gene has 20 exons and spans a genomic distance of 30 kb [20]. In addition to its localisation to the *MEN 2* region, the expression pattern of *RET* suggests it might be a candidate for the *MEN 2* and *FMTC* gene. *RET* is expressed in tissues and cell lineages derived from neural crest including brain, spinal cord, sympathetic and parasympathetic ganglia, thyroid C-cells and adrenal chromaffin cells [21–23]. Further, *RET* is expressed at high levels in tumours of neuroectodermal origin, such as neuroblastoma and the MEN 2-associated tumours, MTC and PC [22–24].

The *RET* proto-oncogene has been implicated in the pathogenesis of another cancer of the thyroid, human papillary thyroid carcinoma (PTC). An oncogenic *RET* isoform (RET^{TPC}) is generated by juxtaposition of the *ret* tyrosine kinase domain to other 5′ sequences in 10–30% of PTC [25–30] (Figure 3). Similar gross alterations of the *RET* gene or the surrounding sequences have not been observed in patients with MEN 2A, 2B or FMTC [16, 31, 32]. The absence of detectable deletions or rearrangements of the *MEN 2* region suggested that the disease mutations might be minute changes or point mutations in *RET*. Sequence analyses of the *RET* gene have demonstrated the presence of very specific missense mutations in patients with MEN 2A, FMTC and MEN 2B (Figure 3).

MEN 2A

In patients with MEN 2A, single base pair substitutions are detected in exons 10 and 11, affecting one of five cysteine residues of the *ret* extracellular domain. Base pair changes in one of codons 609, 611, 618, 620 or 634 result in substitution of any of several amino acids for cysteine residues. The five affected codons lie in a cysteine-rich region close to the ret transmembrane domain (Figures 2, 3). *RET* mutations at one of these five codons are identified in more than 95% of MEN 2A families. The most frequent events, reflecting >85% of all reported cases, are at codon 634 which lies in exon 11 [32–34]. The most frequently observed mutation at that site (>85% of mutations) is a TGC → CGC change that produces a cysteine to arginine substitution [33].

FMTC

FMTC disease mutations are detected in the same exons as for MEN 2A and affect four of the five codons mutated in MEN 2A (611, 618, 620 and 634) (Figure 3). Mutations are detected in >85% of FMTC families and occur most frequently at codons 620 and 634 [33, 34].

MEN 2B

In the case of MEN 2B, a single base pair change in exon 16 within the *ret* tyrosine kinase domain has been identified in >90% of patients [35–37] (Figure 3). Substitution of an ACG for an ATG at codon 918 in these individuals results in substitution of a threonine for methionine. This amino acid change

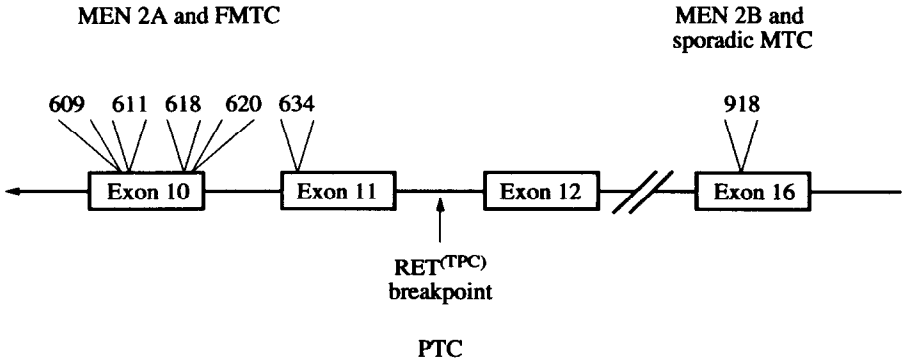

Figure 3. Mutations of the *RET* proto-oncogene in human thryoid cancer. The position of the codons mutated in MEN 2A (609, 611, 618, 620 and 634), in FMTC (611, 618, 620 and 634) and in MEN 2B and sporadic MTC (918) are indicated. The location of the breakpoint involved in *RET* rearrangements found in human PTC is indicated by an arrow.

lies in the substrate recognition pocket of the tyrosine kinase catalytic core, and is predicted to result in altered substrate binding or specificity. The identical mutation at this codon has occurred multiple times in the generation of MEN 2B as evidenced by reports of *de novo* cases of MEN 2B with this mutation [35].

Sporadic tumours

RET mutations of codon 918 also occur somatically in 33–38% of sporadic MTC and in some sporadic PCs [35, 36]. These mutations are identical to those in MEN 2B patients, but occur only in tumour material and are not found in constitutional cells from the affected individual. Conversely, mutations of exons 10 and 11 are very infrequent in sporadic tumours [34, 35], suggesting the MEN 2A and FMTC type mutations can contribute to disease phenotype only when inherited through the germline.

TISSUE SPECIFICITY OF *RET* MUTATIONS

Although there is a degree of overlap in phenotype among MEN 2A, 2B and FMTC, each disease has a distinct and characteristic distribution of tissue involvement (Table 1). By inference then, the pattern of tissues involved in these diseases is dependent on the nature and the position of the underlying *RET* mutation. This hypothesis is supported by comparison of the mutations observed in MEN 2A and FMTC, which may occur in the same codons in exons 10 and 11 (Figure 3). Analyses of disease phenotypes and the position of the mutated *RET* codons suggest a strong correlation between mutation of codon 634 in exon 11 and the presence of PC [33]. Further, a strong correlation is observed between the presence of parathyroid disease and a TGC → CGC substitution at codon 634 which represents a cysteine to arginine change [33].

The correlation between specific *RET* mutations and disease phenotypes suggests that *RET* mutations affect different cells and tissues differently. The relationship between tissue involvement and *RET* mutation may reflect variation in sensitivity of these functions or interactions to different *RET* mutations. The role of ret in thyroid C-cells, for example, may be sensitive to mutation of codons 918, 609, 611, 618, 620 and 634. In adrenal chromaffin cells, only alteration of certain amino acids (634 and 918) has a sufficiently disruptive effect on ret to generate a disease phenotype. In parathyroid, which may be more resistant to ret aberration, only the most disruptive amino acid change at codon 634 (cysteine → arginine) alters ret function enough to result in disease.

NATURE OF THE MEN 2 MUTATION

The inherited cancer syndromes characterised to date arise from the inactivation of tumour suppressor genes. In these cases, predisposition to cancer is conferred by an inherited inactivating mutation in one allele of the disease gene. When a somatic mutation or deletion of the remaining normal allele occurs in such a predisposed cell, tumorigenesis is initiated. Frequently, these events are recognised by loss of constitutional heterozygosity (LOH) in the tumour for the chromosomal region containing the gene of interest. If a similar mechanism applies to *RET* mutations, we would predict that, in tumours from MEN 2 and FMTC patients, the *RET* allele inherited from the normal parent would be deleted or otherwise inactivated in a proportion of cases. Similarly, in sporadic MTC or PC, with somatically acquired *RET* mutations of one allele, the remaining *RET* allele would be inactivated, deleted or rearranged. Several lines of evidence suggest this model does not apply to *RET*. First, allele loss on chromosome 10 is extremely rare in MTC and PC (<5% tumours), despite the frequent occurrence of LOH at other loci in the same tumours [38–42]. Second, pulsed field gel electrophoresis and Southern analyses of the region containing the *MEN 2* locus indicate that neither rearrangement nor deletion of the region occur in tumours or normal tissue from MEN 2 and FMTC patients [16, 31]. Finally, analyses of intragenic *RET* polymorphisms indicate that both copies of the gene are expressed in tumours from *MEN 2* patients, but only a single mutation is detected in *RET* transcripts [32]. Together, these data suggest that the classical tumour suppressor model for inherited predisposition to cancer with inactivation or loss of both alleles of the disease gene may not apply to the *RET* mutations seen in MEN 2.

If *RET* does not conform to the tumour suppressor gene paradigm, it is possible that the *MEN 2* mutations represent inherited activating mutations of *RET*. In this case, the inherited *RET* mutation may result in an altered proliferation potential in thyroid C-cells, adrenal chromaffin cells and/or the parathyroid. The resultant expanded population, while not in itself transformed, provides an increased target population for other transforming mutations. Allele losses on 1p, 3p, 22q and, less frequently, 11p, 13p, and 17p in MEN 2 type tumours, which have been interpreted as progressional events, may have a role in this process [38–40, 42, 43].

In MEN 2B, substitution of a threonine for a methionine at amino acid 918 within the highly conserved substrate recognition pocket of the tyrosine kinase catalytic core may effect an increased activity or, more likely, a change in substrate specificity. The resultant RET isoform may be capable of a broader range of kinase activity, resulting in stimulation of cell proliferation. The mechanism of tumorigenesis in MEN 2A and FMTC, and the role of the cysteine mutations in exons 10 and 11, is less easy to infer. The *RET* extracellular domain contains 28 cysteine residues, 27 of which are conserved between man and mouse [17, 18, 44]. This suggests a functional significance for these cysteines, perhaps in the formation of disulphide bridges necessary for appropriate protein conformation or ligand binding. The cysteines in which MEN 2A/FMTC mutations occur are clustered within a cysteine-rich region close to the transmembrane domain [18] (Figure 3). These amino acids may contribute to inter- or intra-molecular interactions responsible for ret ligand binding or homophilic interactions. Receptor aggregation is a common mediator of ligand binding among tyrosine kinase receptors. Conceivably, the MEN 2A/FMTC mutations produce ret protein conformation changes which promote receptor aggregation in the absence of ligand, leading to ret activation.

Alternatively, *RET* mutations may stabilise receptor dimers, amplifying the effect of normal ligand binding, which again results in increased *RET* function. Although *RET* is a member of the tyrosine kinase receptor family of genes, which includes platelet-derived growth factor receptor [45] and *C-KIT* [46], its ligand and downstream effectors have not yet been identified. Studies are on-going to identify the ret ligand(s) and downstream effector pathways.

RET IN DEVELOPMENT

The expression of *RET* in neural crest derived tissues and tumours has suggested that the gene plays a role in normal developmental processes. In mouse embryo, *in situ* hybridisation has shown that *RET* has a tissue-specific and developmentally regulated pattern of expression, suggestive of a role in neural

crest development or in the cell-to-cell signalling which contributes to neural crest cell migration [47]. This role has been supported by the identification of *RET* mutations in patients with Hirschsprung disease (HSCR).

HSCR is a neurocristopathy characterised by the absence of the enteric parasympathetic ganglia from the hindgut [48]. Infants present with severe constipation, abdominal distension and finally blockage of the colon due to failure of normal peristalsis in the affected portion of the gut [48]. The severity of HSCR is related to the extent of gut involved. HSCR is thought to originate in a defect of neural crest cell migration. Normally, vagal neural crest cells undergo an extensive migration, culminating with population of the gut and formation of the enteric ganglia of the distal colon by approximately 12 weeks gestation [49]. In HSCR, these ganglia are absent.

Recent studies have identified germline mutations of *RET* in patients with sporadic and familial forms of HSCR. The mutations encompass deletions and mutations that clearly result in loss of function, including premature stop codons or alteration of splicing sites [50–53]. Other amino acid changes occur that are also presumed to lead to loss of function. In every case, one normal copy of *RET* remains. Together these data suggest that HSCR arises from a reduced expression of functional ret protein in an early stage of neural crest development. This is supported by the phenotype of *RET* knockout mice (*RET*-/-) which lack any functional ret protein. These mutants have no intestinal parasympathetic ganglia and the kidneys are abnormal or absent [54].

The activating *RET* mutations in MEN 2A, 2B or FMTC may also disrupt normal developmental processes to varying degrees. The presence of the identical codon 918 mutation in MEN 2B patients and a significant minority of sporadic MTC suggests that this mutation can exert its tumorigenic capability in mature thyroid C-cells. However, the same mutation, also acts during development, disrupting neurogenesis and resulting in intestinal ganglioneuromatosis and mucosal neuromas. The scarcity of MEN 2A/FMTC type mutations in sporadic MTC and PCs may indicate that these mutations exert their effects only during a specific developmental window. Thus, the adult C-cell may be resistant to these effects, while the immature C-cell precursor is sensitive to these mutations [35, 36]. Assessment of this model must await the analyses of these *RET* mutations in an animal model system.

DIRECT MUTATION DETECTION: CLINICAL APPLICATIONS AND IMPLICATIONS

While elucidation of disease mutations in *RET* has provided new understanding of its role in development and tumorigenesis, its most immediate application is in management of the familial MTC syndromes. Prior to the availability of screening for MTC, the disease was often not identified until there were widespread metastases, and treatment options were very limited. Screening is now begun as early as 3 years of age [55]. In the past, all individuals at 50% risk for MEN 2A, 2B or FMTC would be offered screening, even though half of the screened individuals would not have the disease mutation. With the advent of DNA mutation analysis, individuals at risk for a familial MTC syndrome can be tested to determine whether they carry the disease mutation present in affected family members [56–58]. The presence or absence of the mutation will dictate which family members require biochemical screening for MTC, PC and/or parathyroid disease. It is important to note that mutation

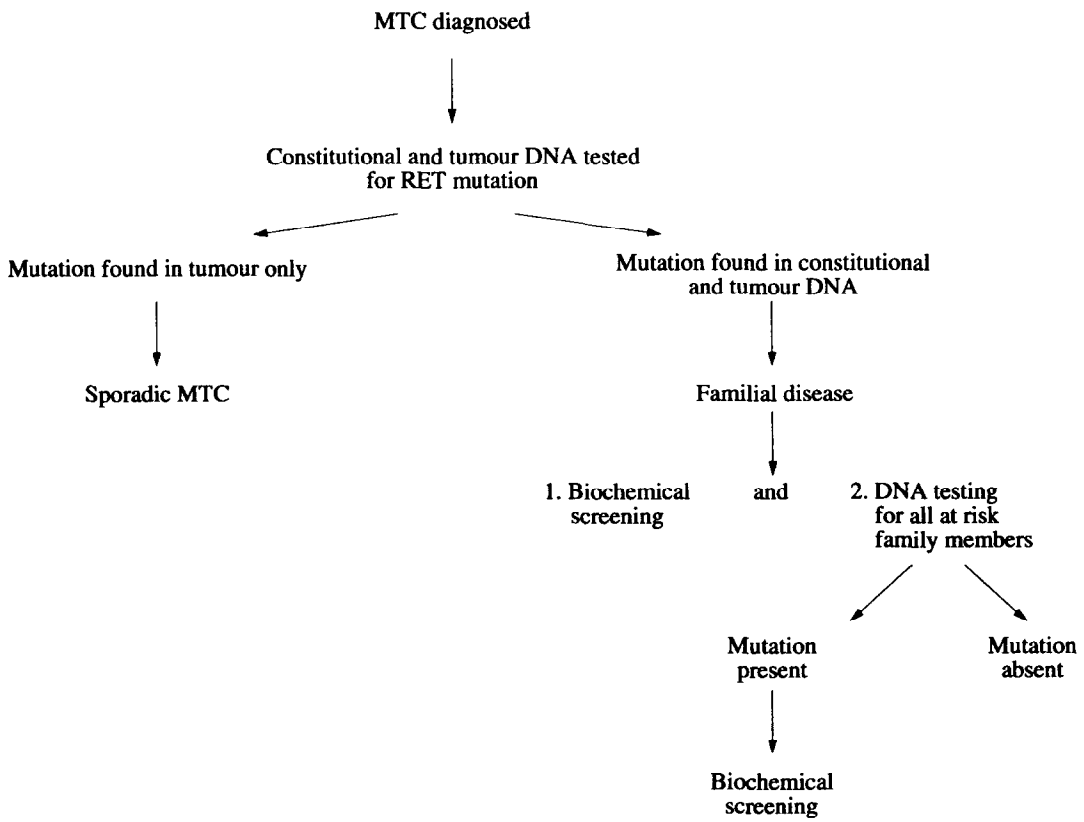

Figure 4. Approach to assessment and screening requirements for individuals diagnosed with MTC.

analysis cannot differentiate between MEN 2A and FMTC [33, 34]. Therefore, any individual found, by DNA testing, to have inherited an MEN 2A/FMTC type *RET* mutation must enter into a biochemical screening programme. The offspring of each person with an identifiable DNA mutation should undergo DNA testing themselves (Figure 4). On rare occasions, a mutation will not be identifiable in affected family members [33, 35–37]. Under those circumstances, mutation analysis cannot be offered to at risk family members.

An individual with apparently sporadic MTC may represent a previously undetected familial case [35–37]. DNA testing can now be used to differentiate truly sporadic cases from familial ones (Figure 4). This requires comparison of the constitutional (e.g. blood) and tumour DNA for mutation of *RET*. If a mutation is identified in tumour DNA only, the MTC is said to be sporadic and biochemical screening of this individual for additional disease features would not be required. If the same mutation is identified in both constitutional and tumour DNA, the MTC represents a new familial case and further biochemical screening is required (Figure 4).

There is now evidence, from long term follow-up of screening programmes for families with MEN 2A and 2B in North America [4, 59] and the Netherlands [7], that early detection and treatment of C-cell hyperplasia can prevent development of MTC. The morbidity and mortality associated with PCs has also been reduced by screening. The identification of the gene responsible for MEN 2A, 2B and FMTC now provides the opportunity for direct mutation testing in these diseases. Since DNA analysis precisely identifies individuals who carry the disease mutation, it will now be possible to improve the efficiency of screening programmes by identifying a high risk population. At the same time, the burden of biochemical screening can be removed from individuals who have not inherited *RET* mutations.

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Neurofibromatosis Type 1

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INTRODUCTION

In 1882, Friedrich von Recklinghausen described a clinical syndrome characterised by nerve-derived tumours called neurofibromas. This syndrome was subsequently termed von Recklinghausen neurofibromatosis, and is now referred to as neurofibromatosis type 1 (NF1) [1,2]. NF1 has been erroneously referred to as the "elephant man" disease from Sir Frederick Treves' description of Joseph Merrick, an Englishman afflicted with a disfiguring disease with features similar to NF1 [3]. While NF1 gained much publicity and notoriety through this misnomer, it is now widely accepted that Merrick instead had proteus syndrome, and the association of NF1 with the "elephant man" is being discouraged [4]. According to current interpretations of literature and ancient artworks, NF1 has been present throughout history [5] and is one of the most frequently occurring human autosomal dominant diseases (1/3500 individuals). This disease is inherited in an autosomal dominant fashion, is found across all ethnic groups and primarily affects tissues derived from the embryonic neural crest.

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