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RESEARCH LETTER

TSH receptor extracellular region mutations in thyroid functioning nodules: further evidence for the functional role of this region in the receptor activation

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Introduction

A causative role for activating mutations of the TSH receptor (TSHR) has been demonstrated in the majority of thyroid hyperfunctioning nodules, as well as for the familial and sporadic congenital nonautoimmune hyperthyroidism [1, 2] and also the rare hyperfunctioning carcinomas [3, 4]. More than 30 different amino acid substitutions have been described so far, and their characterization, together with in vitro mutagenesis studies have been exploited for a structure–function analysis of the receptor, leading to a structural model in which the areas involved in the binding to TSH or signal transduction have been depicted [5]. The emerging picture suggests the existence of a molecular constraint which limits the constitutive activity of the receptor, removed by the interaction of TSH with the extracellular (EX) portion, but with an important role played also by the transmembrane regions in the dimerization process and the functional activation of the receptor [5, 6]. Herein we describe the discovery of two unusual heterozygous mutations occurring in two of seven thyroid functioning nodules detected as single nodules or in a multinodular goiter.

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Materials and methods

Specimen of nodular tissues were collected and immediately frozen after surgical removal from seven patients with single or multinodular hyperfunctioning goiter. Non-nodular normal tissue from the contralateral lobe was also collected. After RNA extraction and cDNA synthesis, PCR amplification of the exons 9 and 10 of the TSHR gene was performed as previously described [3, 4] and the amplified products sequenced with a BigDye Terminator version 3.1 Cycle Sequencing kit in an automated 3130xl analyzer (both from Applied Biosystems, Foster City, CA, USA). The study protocol was approved by the local ethics committee.

Results

Sequencing of TSH receptor gene in a small cohort of seven patients with thyroid hyperfunctioning nodules revealed the presence, in two cases, of unusual somatic heterozygous mutations. The first was a deletion of D403

residue, located in the extracellular domain of the receptor, reported very recently in another case of functioning adenoma and provided in vitro of a strong constitutive activity [7]. The second was a I568F mutation located in the second extracellular loop of the TSHR: mutations of the same residue, but with different aminoacid substitutions (I568T or I568V) have been already reported and analyzed for their increased constitutive activity in vitro [2] (Fig. 1). In both cases, the disease phenotype, including age of appearance, nodular volume, thyroid hormone levels, did not show particular features, different from the other five patients with absence of *TSHR* mutations (data not shown), confirming a major role for additional genetic, epigenetic or environmental modifiers in the phenotypical expression of the disease.

Discussion

Some interesting considerations may be suggested by the present finding regarding the structure–function relationship of the TSHR. The D403 deletion detected in our first patient occurs in the cysteine-box3 of the hinge region of the receptor; interestingly, constitutive activation of the

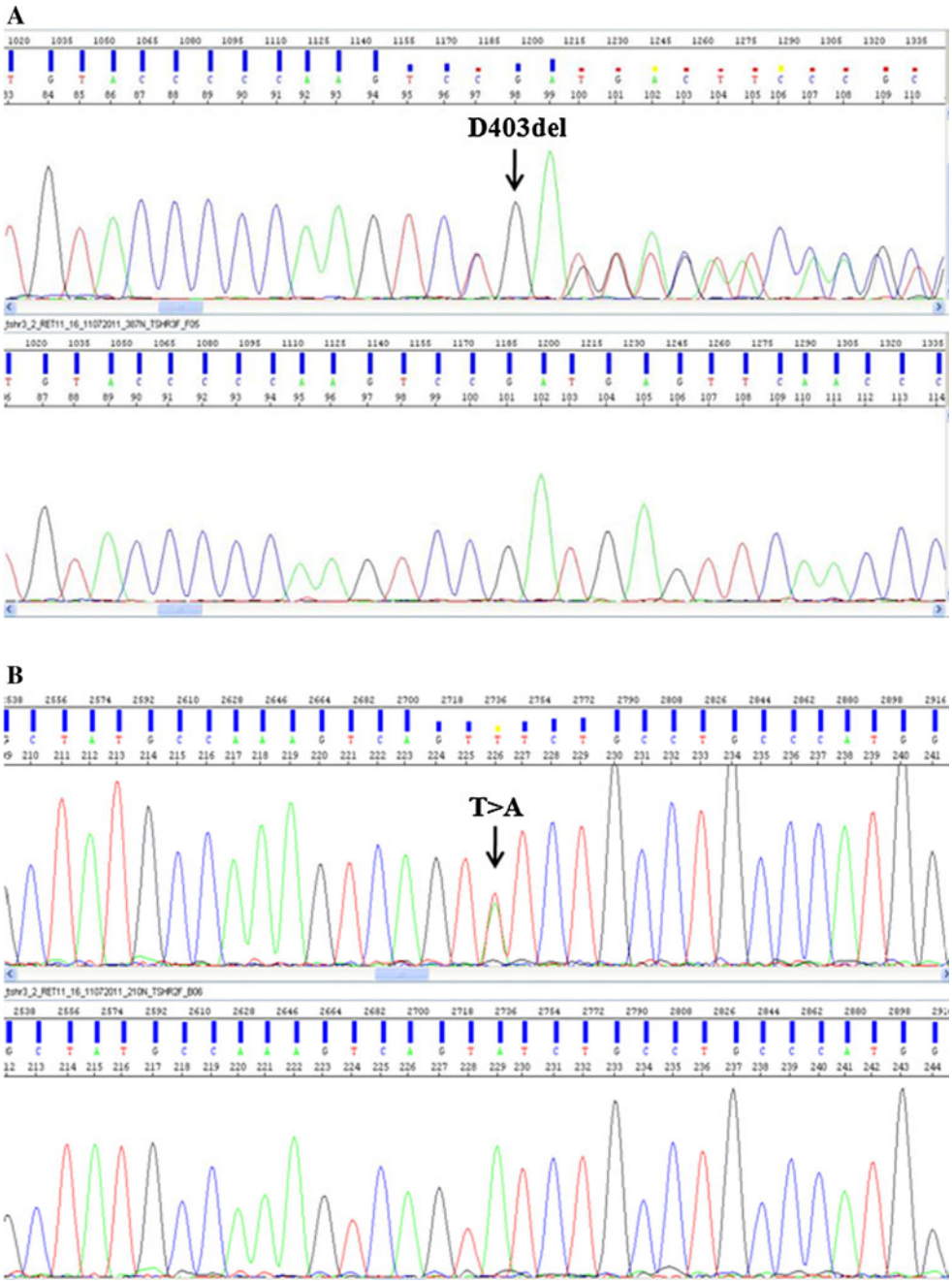

Fig. 1 TSH receptor mutations detected in two thyroid hyperfunctioning adenomas, using direct sequencing of the entire exon 10 of the gene. **a** Deletion of codon 403 (D403del). **b** T>A substitution causing phenilalanine for isoleucine substitution at codon 568

TSHR has been reported for point mutations occurring in four additional aminoacid residues of the same area (P400, E404, N406, and P407). Moreover, a mutation in the same site has been described in a case of congenital hypothyroidism [8], and other inactivating mutations were found in the negative charged aminoacids (E409 and D410) located in the same region. Thus, the integrity of such a region may be necessary for the switching process between basally active and further activated conformation [6]. As concerning the second mutation detected in this series of toxic nodules, it has been proposed a TSHR model in which a direct interaction occurs between I568 in the second EX loop and I640 located in the sixth transmembrane region, suggesting a dynamic interface between these two portions of the receptor, involved in the constraint of the receptor basal activity and regulation of different conformation [9]. This hypothesis is strongly supported by the detection of many pathogenic mutants in these regions, including three different aminoacid substitutions in the same residue of the second EX loop (the third detected in the present study).

Altogether, these observations emphasize the role of the EX region (including the loops) in the functional activity of the TSH receptor, and its potential pathogenicity related with its weak constrain status. Indeed, in addition with mutational alterations of the binding sites for TSH, responsible for congenital hypothyroidism, the EX region may also host activating mutations, as detected in sporadic or familial hyperthyroidism [2]. Furthermore, dissociation between binding activity and signal transduction activity has been described for mutations in this region detected in two families [10, 11], and confirmed by in vitro studies [12].

Collection of additional mutational data from novel series of samples, associated with mutagenesis in vitro studies, when combined with novel physicochemical approaches for direct probing of the structural changes may add novel tasks in the puzzling issue underlying the mechanism of activation of the TSH receptor.

Conflict of interest The authors declare that they have no conflict of interest.

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