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Search for Abnormalities of Nuclear Corepressors, Coactivators, and a Coregulator in Families with Resistance to Thyroid Hormone without Mutations in Thyroid Hormone Receptor β or α Genes*

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ABSTRACT

The syndrome of resistance to thyroid hormone (RTH) is characterized by decreased tissue responsiveness to thyroid hormones. Inheritance is usually autosomal dominant due to mutations in the ligand-binding domain or adjacent hinge region of the thyroid hormone receptor β (TRβ) gene. Six of 65 families with the RTH phenotype studied in our laboratory had normal TRβ1 and TRβ2 gene sequences. Their clinical characteristics were not different from those of subjects with TRβ gene mutations. Four of the 6 families were amenable to linkage analysis, and TRα involvement was excluded. Candidate genes were then evaluated for their possible involvement in the RTH phenotype in these 4 families: 2 coactivators [NCoA-1 (SRC-1) and NCoA-3 (AIB-1)], 2 corepressors (NCoR and SMRT), and a coregulator (RXRγ). DNA was obtained from 8 affected subjects and 41 of 45 living first degree relatives. In 2 of the 4 families, the mode of inheritance could be determined by pedigree analysis and was found to be autosomal dominant. Linkage analyses were performed using polymorphic markers near or within the 5 candidate genes. When analyses were not informative or linkage could not be excluded, direct sequencing of the genes in question was performed.

Involvement of NCoA-1 was excluded in all four families assuming autosomal dominant inheritance. Roles for NCoR, SMRT, and NCoA-3 were excluded in three and a role for RXRγ was excluded in two of the four families. However, if the two families without proven dominant mode of inheritance were compound heterozygous, only the involvement of NCoA-1 could be excluded in both. Roles for NCoR, SMRT, and RXRγ were excluded in one of these two families. Thus, NCoA-1 and RXRγ genes were not found to be the cause of RTH in subjects without TR gene mutations even though the absence of NCoA-1 and RXRγ is the cause of RTH in mice. Involvement of other candidate genes in the mediation of thyroid hormone action as well as intracellular hormone transport needs to be explored in these families with non-TRβ, TRα RTH. (*J Clin Endocrinol Metab* **85:** 3609–3617, 2000)

THE SYNDROME of resistance to thyroid hormone (RTH) is characterized by decreased tissue responsiveness to thyroid hormones (1, 2). Since its first description in 1967 (3), more than 700 individuals with RTH in 250 families have been identified (4) (our personal observations). Clinically, these patients have persistent elevation of serum levels of free T_4 and T_3 with nonsuppressed TSH. Most have goiter and sinus tachycardia, and some have associated learning disabilities (1, 5). In 1989 and 1990, the first two mutations in the ligand-binding domain of the thyroid hormone receptor β (TRβ) gene associated with the syndrome, G345R and P453H, were reported by Sakurai *et al.* (6) and Usala *et al.* (7), respectively (see also Ref. 8). In the majority of subjects, RTH is dominantly inherited. Autosomal recessive inheritance due to complete deletion of the protein-coding region of the TRβ gene was found in only one family (9). All mutations linked to RTH are located in the ligand-binding domain and adjacent hinge region of the TRβ gene, in three clusters (5, 10–14).

Mutations in the TRβ gene were not found in a subgroup of patients with RTH. We first described one such family in 1996 (15), and more recently, we reported 5 additional families (16). They represent approximately 10% of the 65 families with RTH studied in our laboratory. In addition to having normal TRβ1 and TRβ2 genes, as determined by sequencing, involvement of the TRβ and TRα genes was excluded by linkage analysis in 2 and 4 of these 6 families, respectively (15, 16). Their clinical phenotype was similar to that of individuals with RTH due to TRβ gene mutations and has been previously described in detail (15–18).

TRs homodimerize or form heterodimers with retinoid X receptors (RXRs) and bind to specific DNA sequences, termed thyroid hormone response elements (TREs). In the absence of T_3, TR homodimers and heterodimers are associated with corepressors (NCoR and SMRT) that repress or

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silence the transcription of genes positively regulated by the ligand. Binding of T_3 to TRs releases the corepressors and recruits nuclear coactivators, such as NCoA-1 (SRC-1), NCoA-2 (SRC-2/TIF-2/GRIP-1), and NCoA-3 (SRC-3/pCIP/AIB-1), which stimulate gene transcription (19). Mutant TRβs interfere with the functions of the wild-type TRs, a phenomenon termed the dominant negative effect (DNE) (20). The DNE involves the occupation of a TRE by a mutant TR that cannot bind T_3 or has reduced affinity for the ligand, tighter affinity for the corepressors (21–23), or reduced ability to recruit coactivators (24, 25) necessary to enhance gene transcription. For DNE to occur, TR has to bind to TRE, which explains why no mutations have been identified in the DNA-binding domain.

In the absence of TRβ mutations, a RTH phenotype could theoretically be caused by abnormal corepressors, coactivators, or coregulators that have altered interaction with TR. This was recently found to be true in mice deficient in SRC-1, which, in addition to sex hormone resistance, manifested the phenotype of RTH (26). Additionally, RXRγ-deficient mice were found to have biochemical changes consistent with mild RTH (27).

In this study we explored the possibility that a defective cofactor or RXRγ may be the cause of RTH in individuals without mutations in TRβ or TRα genes. Of the six families we have previously reported (15, 16), the pedigrees of four were potentially amenable to linkage analysis because more then one family member was affected, or affected subjects had sufficient number of unaffected siblings and progeny. We performed linkage studies for two corepressors (NCoR and SMRT), two coactivators (NCoA-1 and NCoA-3), and RXRγ. When the linkage to one of these genes could not be excluded or the result of linkage analysis was not informative, the gene in question was sequenced. We were able to show that expression of the RTH phenotype in these four families does not involve a defect in SRC-1. The involvement of NCoR, SMRT, and NCoA-3 was excluded in three and the involvement of RXRγ was excluded in two of the four families.

Subjects and Methods

Subjects

Members of four families were investigated. Their clinical presentation and features were previously described in detail (15–18). Family Mma (Fig. 1), previously reported as family F25, and family Mal (Fig. 2) each have three affected individuals. The inheritance pattern is auto-

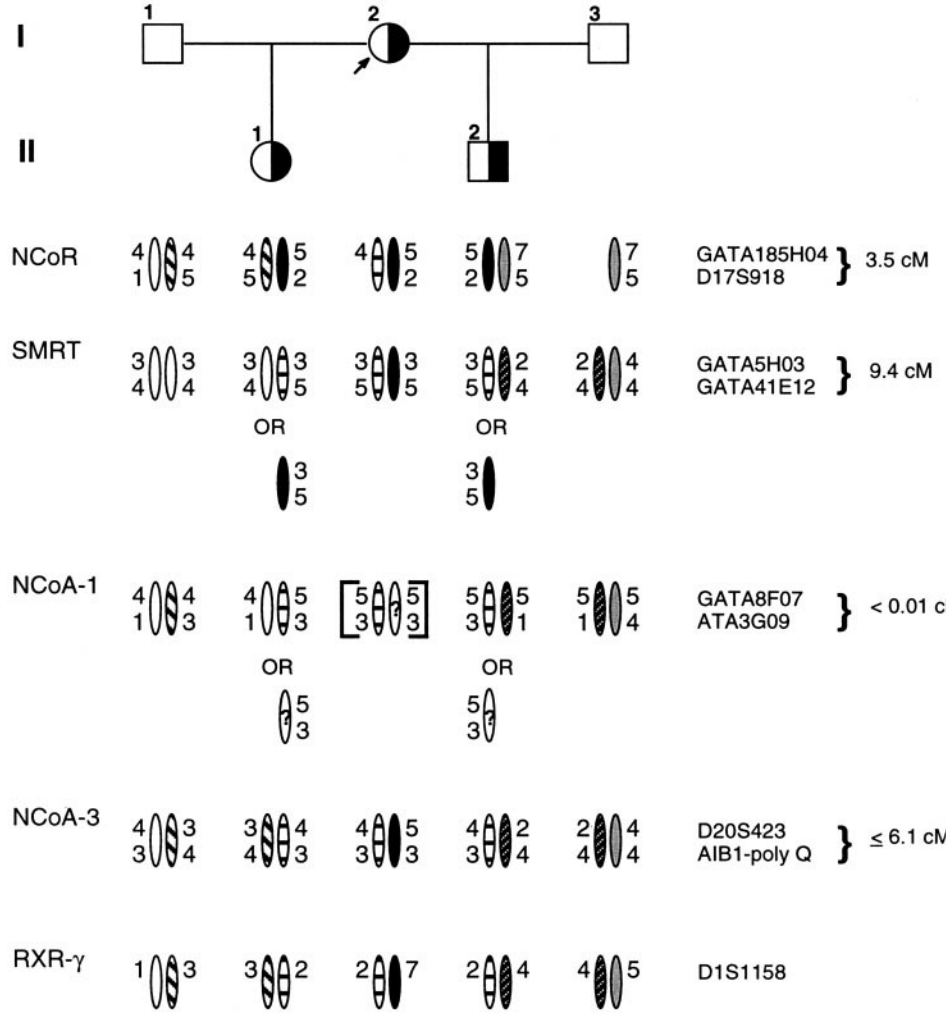

FIG. 1. Pedigree of family Mma showing the phenotype and haplotypes for markers that type two corepressors (NCoR and SMRT), two coactivators (NCoA-1 and NCoA-3), and the RXRγ genes. Affected subjects have *half-black symbols*, and the *arrow* points to the proposita. Haplotypes are aligned with each individual symbol, and *shading* is provided to help trace the inheritance of the different alleles. For individuals from whom no samples were available, haplotypes are deduced, when possible, using results obtained from relatives. These are enclosed in *brackets*. Markers are identified by name and distances between markers as indicated. For detailed description, see *Subjects and Methods*.

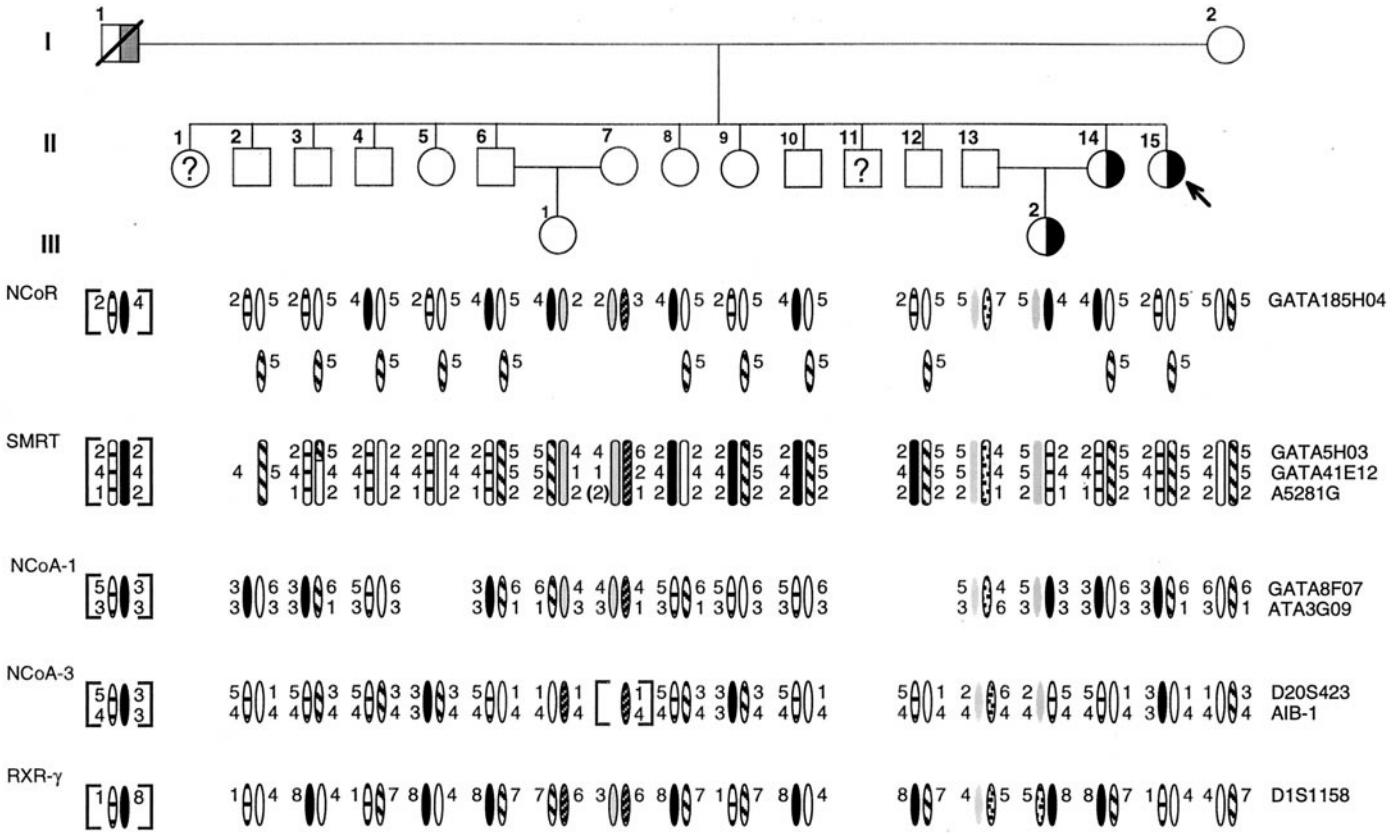

FIG. 2. Pedigree of family Mal showing the phenotype and haplotypes for markers that type two corepressors (NCoR and SMRT), two coactivators (NCoA-1 and NCoA-3), and the RXRγ genes. For details, see Fig. 1. Subjects with unknown phenotype because they were not tested are indicated by a question mark (?). The presumably affected subject I-1 is indicated by a *shaded symbol*.

somal dominant. Family Mch (Fig. 3) and family Msn (Fig. 4), each have one affected individual with normal siblings and progeny (16). All affected individuals displayed the clinical features characteristic of RTH with elevated free T_3 and T_4 and nonsuppressed TSH. Furthermore, in three of the four families (Mma, Mal, and Mch), reduced sensitivity to T_3 in central and peripheral tissues was documented by the response to administration of supraphysiological doses of the hormone (16–18).

Blood samples were obtained from most first degree relatives. In fact, of 52 family members, 5 were dead, and samples were obtained from all living members except for 9. Five of these belonged to family Mma, and previous thyroid function tests have shown that they (both parents and all siblings) were not affected (17); 2 were siblings of the proposita of family Mal, 1 was a sibling of the proposita of family Mch, and one was a spouse of the proposita of family Msn. Genomic DNA was extracted from circulating white blood cells by a standard technique (28). The study protocols were approved by the institutional review board, and informed consent was obtained from all individuals who participated in the study.

Genotyping of the NCoR gene

At the time of the study, the location of the human NCoR gene was not known. We obtained human NCoR genomic clones containing the 3′-fragment from Kathryn B. Horwitz and Jennifer K. Richer, University of Colorado Health Sciences Center (Denver, CO). Partial sequencing in our laboratory provided the 3′-untranslated nucleotide sequence. This allowed the synthesis of oligonucleotide probes to screen a G3 radiation hybrid library (Research Genetics, Inc., Huntsville, AL). The forward primer was 5′-CATCTGTGGGCTGGCTCTCCT-3′, and the reverse primer was 5′-CATTGCTCTCAGCACAGTACGA-3′. PCR was performed in a volume of 12 μL with 8 μmol/L of each primer, buffer containing 2.5 mmol/L Mg, 10 nmol/L deoxy (d)-NTPs, 10% dimethylsulfoxide, and 0.2 U *Taq* DNA polymerase. The PCR product had the expected size of 183 bp. The PCR conditions were denaturation at 94 C for 1 min, annealing at 60 C for 1 min, and extension at 72 C for 20 s for a total of 35 cycles. The results of the library screen were submitted to the Stanford Radiation Hybrid Mapping Program for two-point maximum likelihood analysis. Linkage to marker AFMb357yg9, located on chromosome 17, had a logarithm of odds score of 14.46. For a clear separation of polymorphic bands, genotyping was carried out using the tetranucleotide marker, GATA185H04, located 1.6 centimorgans (cM) from AFMb357yg9. The location is within the broader area of chromosome 17, reported previously (29). For family Mma, we used a second marker, D17S918, because of technical difficulties in identification of the proposita's genotype using marker GATA185H04. The distance between the latter marker is 3.5 cM from GATA185H04 and 1.9 cM from AFMb357yg9.

Genotyping of the SMRT gene

Although at the time of the study it was known that the SMRT gene is on chromosome 12, its exact location had not been identified. We screened a G3 radiation hybrid library using an oligonucleotide primer complimentary to the 3′-untranslated region of the SMRT gene. Eight micromoles per L of the forward primer 5′-AGAGACCTTACTCAGGGGAT-3′ and the same amount of the reverse primer 5′-CTGACTTGGTTTCCAGCAAT-3′, to yield a PCR product of 334 bp, were used in a PCR performed in a volume of 12 μL with buffer containing 2.5 mmol/L Mg, 10 nmol/L dNTPs, 10% dimethylsulfoxide, and 0.2 U *Taq* DNA polymerase. The PCR conditions were denaturation at 94 C for 30 s, annealing at 58 C for 1 min, and extension at 72 C for 1 min for a total of 35 cycles. The results of the library screen were submitted to the Stanford Radiation Hybrid Mapping Program for two-point maximum likelihood analysis. The linkage to the marker GATA5H03, located on chromosome 12, had a logarithm of odds score of 9.79. A second marker, GATA41E12, was also selected for genotyping. The distance between the

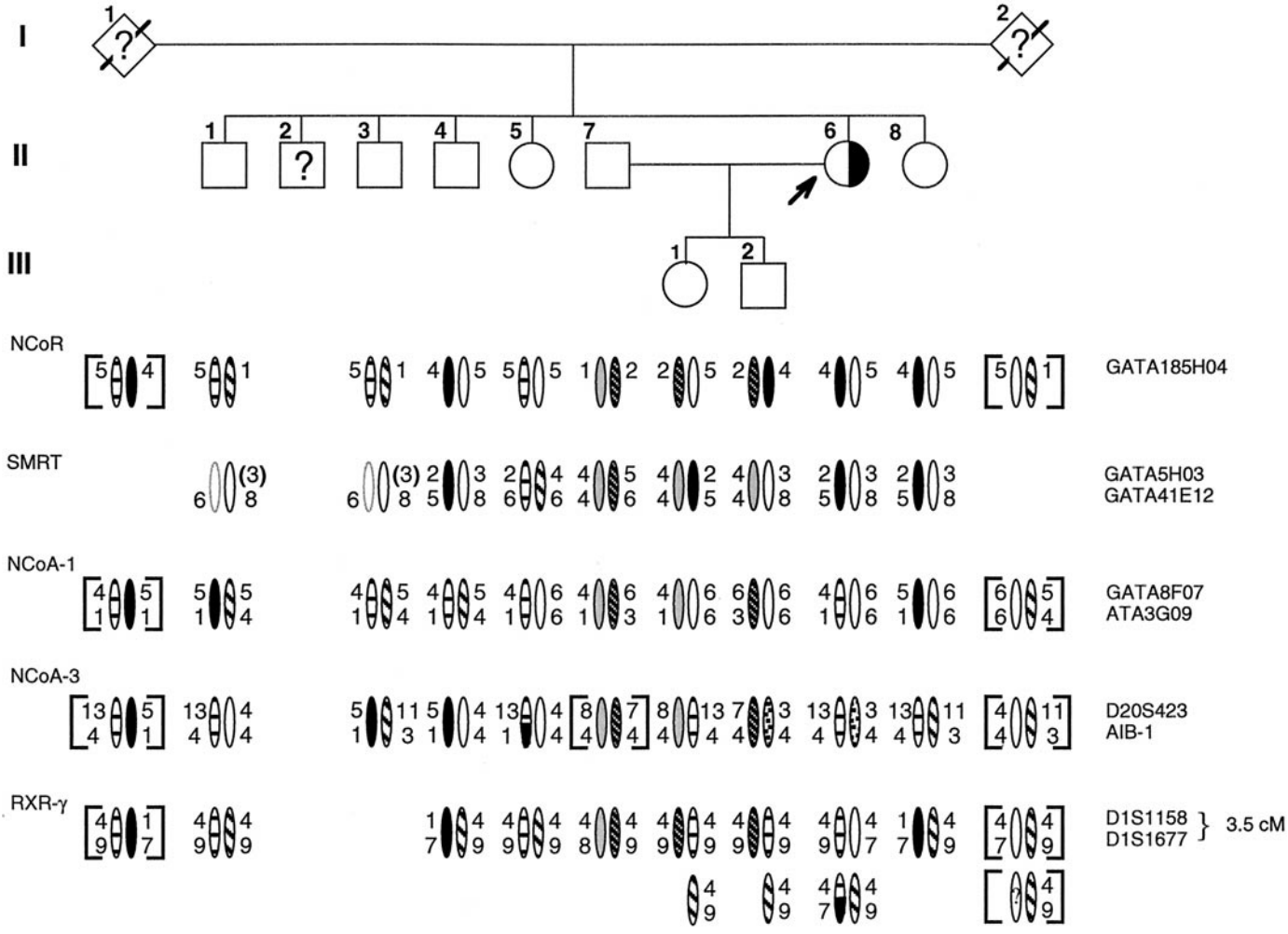

FIG. 3. Pedigree of family Mch showing the phenotype and haplotypes for markers that type two corepressors (NCoR and SMRT), two coactivators (NCoA-1 and NCoA-3), and the RXRγ genes. For details, see Figs. 1 and 2.

two markers is 9.4 cM. In addition, we identified a single nucleotide polymorphism in the 3′-untranslated region of the gene [A/G at nucleotide 5281 (SMRT-A5281G)]. This creates a unique restriction site for the enzyme *Bsa*WI. Digestion of the 334-bp DNA fragment, amplified with the primers used for the screening of the radiation hybrid library, produced a 282-bp fragment in the presence of G at position 5281, which was detected by separation on a 2% agarose gel. The 334- and 282-bp fragments were designated 1 and 2, respectively. Genotyping using this intragenic marker was performed in family Mal in addition to the above two markers.

Genotyping of the NCoA-1 gene

The NCoA-1 gene has been mapped to chromosome 2. Two markers, ATA3G09 and GATA8F07, located less than 0.01 cM apart, were used for genotyping. Both are within 1.1 cM of the NCoA-1 gene.

Genotyping of the NCoA-3 gene

The NCoA-3 gene is located on chromosome 20. We used the intragenic polymorphism in the polyQ region of the coding sequence (AIB1-polyQ) as previously reported (30). A second marker, D20S432, located within 6.1 cM of the NCoA-3 gene was also used for genotyping.

Genotyping of the RXRγ gene

The RXRγ gene has been mapped to chromosome 1. The marker D1S1158, located within 4.1 cM of the RXRγ, was first selected. Due to technical difficulties and failure in some instances to obtain informative results with this marker, the markers D1S1677 and ATA38A05 were also used. D1S1677and D1S1158 are 2.8 and 0.7 cM on either side of ATA38A05, respectively. RXRγ has been mapped to a region of 3.4 cM spanning the markers D1S1677 and ATA38A05, and thus is within 1.5 cM of at least one of the three markers.

Methods for marker identification

With the exception of SMRT-A5281G and AIB1-polyQ, all polymorphic markers were purchased from Research Genetics, Inc. as fluorescence-labeled oligonucleotides. PCRs were performed in a 5-μL volume with buffer containing 2.5 mmol/L Mg, 10 mmol/L dNTPs, 8 μmol/L of each primers, and 0.2 U *Taq* DNA polymerase. The fluorescent PCR products were separated using an automated sequencer (ABI 377, Perkin-Elmer Corp., Foster City, CA), visualized, and printed.

Sequencing of the SMRT gene

SMRT complementary DNA (cDNA) from fibroblasts of the proposita of family Mma along with cDNA from 3 normal, unrelated individuals were sequenced. Primer sequences are shown in Table 1. PCR was performed in a volume of 100 μL with 8 μmol/L of each primer and buffer containing 2.5 mmol/L Mg, 10 nmol/L dNTPs, and 0.2 U *Taq* DNA polymerase. The conditions were denaturation at 94 C for 1 min, annealing at the temperature indicated in Table 1 for 1 min, and extension at 72 C for 1 min for a total of 35 cycles. All PCR products were

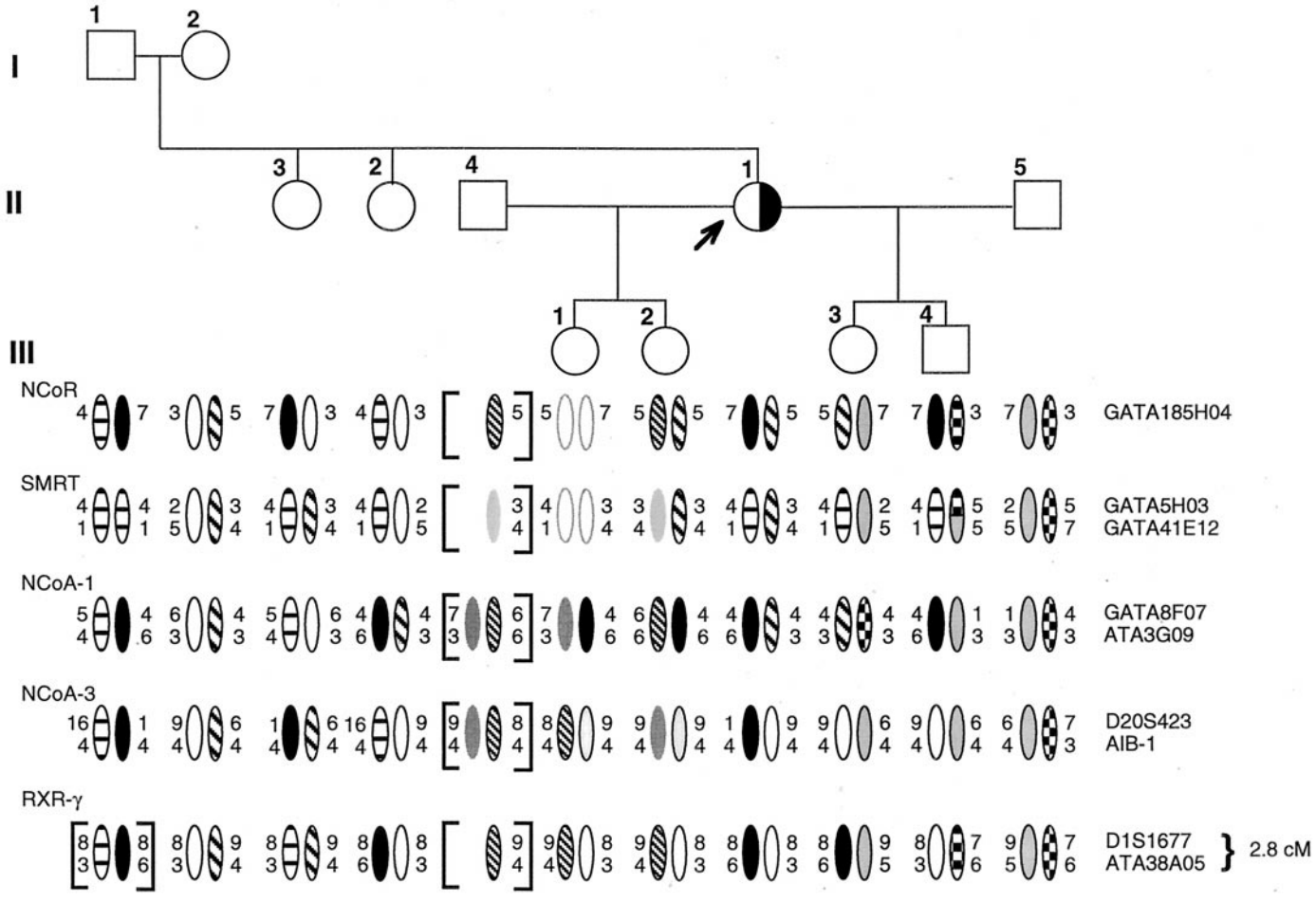

FIG. 4. Pedigree of family Msn showing the phenotype and haplotypes for markers that type two corepressors (NCoR and SMRT), two coactivators (NCoA-1 and NCoA-3), and the RXRγ genes. For details, see Figs. 1 and 2.

purified by electrophoresis on a low melting agarose gel and sequenced using an automated fluorescence based sequencer (ABI 377, Perkin-Elmer Corp.).

Sequencing of the NCoA-1 cDNA

NCoA-1 cDNAs of the propositi of families Mma and Mch were sequenced. Messenger ribonucleic acid was extracted from Epstein-Barr virus-transformed circulating lymphocytes obtained from the affected subject of family Mch (31). Cultures of skin fibroblasts collected from affected members of families Mma and Mch were also used as a source of messenger ribonucleic acid. cDNA was prepared using a RT system (Promega Corp., Madison, WI).

The entire cDNA sequences of the two splice variants (32) were amplified using the primers listed in Table 2. PCR was performed in a volume of 100 μL with 8 μmol/L of each primer and buffer containing 2.5 mmol/L Mg, 10 nmol/L dNTPs, and 0.2 U *Taq* DNA polymerase. The conditions were denaturation at 94 C for 1 min, annealing at the temperature indicated in Table 2 for 1 min, and extension at 72 C for 1 min for a total of 35 cycles. The sizes of the PCR products are shown in Table 2. All were purified by electrophoresis using low melting point agarose gel and sequenced (ABI 377, Perkin-Elmer Corp.).

Results

The haplotypes of markers adjacent to or within the four cofactors and RXRγ genes are shown in Figs. 1–4 and results are summarized in Table 3.

Family Mma

In this family, the pattern of RTH transmission is consistent with autosomal dominant inheritance. Furthermore, the proposita probably has a new mutation, as neither parent and none of five siblings (not shown in Fig. 1) expressed the RTH phenotype (15, 17). The study for linkage to SMRT was not informative because the proposita is homozygous for both markers. We could not use the intragenic marker SMRT-A5281G because the proposita was also homozygous (G/G) for this marker. Partial sequence of SMRT cDNA of the proposita was normal (nucleotides 722-3388, 3700–4069, 4081–4539, 4620–5005, 5060–5466, and 5470–5826; coding sequence spanning from nucleotides 496-4984). We found two differences in our sequences from the published sequence (33) that produced amino acid changes. In the proposita and two unaffected and unrelated individuals, nucleotide 2114 was a C rather than a T (M705T), and nucleotide 3110 was a C rather than A (K1037T).

Linkage of RTH to NCoA-1 in this family could not be completed due to the inability to genotype the proposita despite repeated attempts. Based on the haplotypes of the two children (II-1 and II-2), it is likely that the analyses would have not been informative. However, we were able to ex-

TABLE 1. Oligonucleotide primers used for the amplification of the SMRT cDNA

Fragment[a]	Primers[b]	Size of product (bp)	Annealing temperature (C)
1	5′-GTAATGATCCGATCTTCGGATCCTTCTAA-3′ 5′-CATGGAGGCCCAGGGGCAGTGGGTGACCA-3′	685	60
2	5′-GTGAGGTGATCAAGGCCTCCCCGCATGCCC-3′ 5′-GGCCCTTCTTGCCTTCGTAGATGACGTGGC-3′	648	60
3	5′-CATGGACCCCAAAAAGCTGGCACCCT-3′ 5′-GCCCTCCCGCTTTAGGAGCTTGGCCT-3′	684	60
4	5′-CTCAAAGAGCAGCACCACATCCGCGGGTC-3′ 5′-GCTCCCGCATGGTCACGGGCGAACCTCGT-3′	663	60
5	5′-GGCCAGGGACCGCCAGCAGCTCG-3′ 5′-CAGCATATCAGCTCGCTGGGCCAT-3′	601	57.5
6	5′-CTACCCGCACCTGTACCCACCCTACCT-3′ 5′-CTCCAGATGGGTGCGTGCTCCACCGTC-3′	506	60
7	5′-GGGATCGGGAGCGGGAAAAGTCCATCCT-3′ 5′-GTGCGGGCGATGGTGGCGTGGCCAGAGA-3′	576	57.5
8	5′-CTCGATGGGGTCTACCCTACCCTCA-3′ 5′-GTGACCTTTGACCCCTGGGGCGGTC-3′	617	57
9	5′-GCAGCCAGGCCCCGTGAAGCTTG-3′ 5′-CTTGGACTTGACCATGGCGGAGT-3′	594	60
10	5′-GCAAGAGGTCTCCAGAGCCAAACAA-3′ 5′-TGTGGTCACTCCGTCCGTCAGCA-3′	572	60
11	5′-GCAGGAACATGCCAGCACCAACAT-3′ 5′-CCTTCCCGAGCACCAGGTAAACAT-3′	809	60
12	5′-GACAGCGAGTGACTCAGAACAGGGC-3′ 5′-CTGGGGCAGTCTGTGGGGACCGGAACC-3′	508	60
13	5′-CGCCCTCCCTCCGCCTCCCATCCCGCTTA-3′ 5′-GGAGTATAATTCGCTTTTTAATTAGAAC-3′	580	55

[a] All amplified fragments are contiguous and overlap.
[b] The sequence listed first is that of the forward primer of each pair.

clude a mutation in the coding sequence of NCoA-1 in this family by direct sequencing of the proposita's cDNA. NCoR, NCoA-3, and RXRγ show possible linkage to the RTH phenotype, as all affected individuals (I-2, II-1, and II-2) share a common allele. Unfortunately, we also encountered technical difficulties with the genotyping of RXRγ using markers D1S1677and ATA38A05.

Family Mal

Pedigree analysis revealed that the RTH phenotype in family Mal is inherited dominantly. Linkage analyses exclude the involvement of NCoR, NCoA-1, NCoA-3, and RXRγ in the expression of the RTH phenotype. The haplotypes derived from the combination of three markers (SMRT-A5281G, GATA5H03, and GATA41E12) also exclude SMRT involvement. Note that there is a recombination of the maternal allele in subject II-3, which is not unexpected given the distance of 9.4 cM between markers GATA5H03 and GATA41E12.

Family Mch

The mode of inheritance of RTH in family Mch cannot be determined from the pedigree, because only one family member is affected. The involvement of NCoR and SMRT was excluded assuming the syndrome is dominantly inherited, as the proposita transmitted both alleles to each of her unaffected children. In addition, the proposita shares common NCoR alleles with three unaffected siblings (II-4, II-5, and II-8). Involvement of NCoR and SMRT was excluded should the inheritance be recessive (compound heterozygous),[1] because the proposita has the same haplotype as two unaffected siblings (II-4 and II-8).

Linkage analysis excludes the involvement of NCoA-1 regardless of whether inheritance was dominant or recessive based on shared alleles between the proposita and an unaffected sibling (II-5). However, linkage cannot be excluded if the proposita had a neomutation of NCoA-1, because she passed the same allele to both of her children. Nevertheless, sequencing of NCoA-1 cDNA from the proposita showed no abnormalities. Linkage of RTH to NCoA-3 is also excluded if the inheritance mode is dominant, because the proposita passed each of her two alleles to her unaffected children. However, if the syndrome is recessively inherited, linkage to NCoA-3 cannot be ruled out, because none of the normal siblings shares the haplotype of the proposita.

The presence of a fifth allele for NCoA-3 in generation II of the family, which is unique in the proposita, is suggestive of nonpaternity or a mutation. As both parents are deceased, their haplotypes could only be deduced from data derived from their progeny, and therefore, establishing paternity is not simple. In light of this, we typed 8 additional highly polymorphic markers, bringing the total number studied to 13. Only one, identified in the haplotype of NCoA-1, had a transmission pattern suggesting nonpaternity in the

[1] Throughout this communication, the use of the term recessive applies to compound heterozygous.

TABLE 2. Oligonucleotide primers used for the amplification of the NCoA-1 cDNA

Nucleotide	Size of product (bp)	Primer[a]	Annealing temperature (C)
−88 to 414	502	5′-GCTACCCTCTGGAACTCAAGATTT-3′ 5′-CTCTGACACAAATACAATTCTC-3′	58
336 to 963	628	5′-GGGACCTCTTCTTTTGGAGGCT-3′ 5′-GCCACGAGTCATCACTTCTTGG-3′	64
867 to 1261	395	5′-AGATTTAGTGAGGAAGTGC-3′ 5′-GGCGGTTTATTCTGGTGGATAC-3′	52
1179 to 1563	385	5′-TATCTCTCCAGCTCATGGTGTG-3′ 5′-CATGCCAACGGGAGAGCTTA-3′	58
1479 to 1870	392	5′-TACTTCTGGATTGGCAACAAG-3′ 5′-TGTCTCCATCTGAAAGTCTGTC-3′	58
1794 to 2172	379	5′-TGACAACAGCTCTAGTGATGGC-3′ 5′-CTGTCCAGTCACTGACACAGA-3′	52
2068 to 2495	428	5′-CTACACCGGCTCTTACAGGAG-3′ 5′-TCACATAAGCCTGGCAACTGTG-3′	52
2429 to 3165	737	5′-ACCTTGACCAGTTTGATCAGTTAC-3′ 5′-TCGAAGCTGGTTAGGTGCAGC-3′	65
3097 to 3759	663	5′-TCACCTGGCATGGGCATGCAG-3′ 5′-GCTCCCAGGGCTTAGAGAT-3′	55
3688 to 4091	404	5′-TTCCAGTATCCAGGAGCAGGA-3′ 5′-GTGTGTCTCAGTGCGGGATCA-3′	58
3982 to +125	470,524[b]	5′-GTTCAGAACATGAACCCA-3′ 5′-CTCCAAATGCTACGACCTGA-3′	50
4108 to +3	222,276[b]	5′-CAGCTCTCATCCACTGACCTTCTC-3′ 5′-TTGTTATTCAGTCAGTAGCTGCTG-3′	68

[a] The sequence listed first is that of the forward primer of each pair.
[b] Two splicing variants.

proposita (II-6). In simulation studies in which a half-sibling is generated in a pedigree of five full siblings, more than 20% of replicates had none or only 1 incompatibility. This proportion increased as the marker was assumed to be less informative. Thus, we have little power to determine whether the incompatible type at D20S423 in subject II-6 is due to nonpaternity or a mutation. This result has little effect on the overall interpretation of data. Indeed, assuming nonpaternity, linkage of a dominantly inherited phenotype to NCoR, SMRT, and NCoA-3 can still be excluded based on the haplotype of her 2 unaffected children alone, as indicated above. A recombinant allele in subject II-5 for the 2 NCoA-3 markers, located 6.1 cM apart, is not unusual.

The haplotypes of markers for RXRγ cannot be deduced unequivocally. One parental allele inherited by the proposita depends on whether one of her alleles inherited from the other parent was a recombinant (see Fig. 3). In either event, linkage cannot be excluded. Unfortunately, typing with marker ATA38A05 could not be accomplished.

Family Msn

Linkage of the RTH phenotype to all four cofactors and RXRγ was excluded in this family, assuming a dominant mode of inheritance. However, if the proposita had a neomutation, we cannot exclude NCoA-3 involvement, because she gave the same allele to all four of her phenotypically normal children. If RTH in this family were recessively inherited, the linkage data exclude NCoA-1 and RXRγ involvement, but not NCoR or NCoA-3, because the unaffected siblings (II-3 and II-2) do not share the same haplotype with the proposita. The results of linkage analysis for SMRT were not informative for a recessive inheritance, because both alleles of the father of the proposita (I-1) have an identical haplotype, making it impossible to determine whether the proposita shares the same haplotype with her unaffected sister, II-3.

Discussion

In this study we explored the roles of two corepressors (NCoR and SMRT), two coactivators (NCoA-1 and NCoA-3), and a coregulator (RXRγ) in the expression of the RTH phenotype in eight subjects with no mutations in the TRβ gene. These subjects belong to four unrelated families in which, in addition to TRβ gene sequencing, linkage analyses have excluded the involvement of the TRβ gene in two (Msn and Mch) and the TRα gene in all four (15, 16). The phenotype of these subjects was not different from that of individuals with RTH due to TRβ gene mutations (15–18).

The dominant inheritance in the majority of cases with RTH is due to interference of the mutant TRβs with the functions of the wild-type TRs. This DNE requires that the mutant TRs 1) bind to DNA at promoter sequences of target genes (34), 2) homodimerize (35) and heterodimerize with RXR (36), 3) interact with corepressors (22), and 4) recruit coactivators (14, 25).

Although in most instances the severity of RTH correlates with the degree of T_3 binding impairment of the mutant TRβ (37, 38), discrepancies appear to be due to a strong DNE caused by abnormal interactions with DNA, coactivators, corepressors or RXR. For example, the mu-

TABLE 3. Summary of results based on linkage analysis and gene sequencing

Family	Cofactors	Dominant inheritance	Recessive inheritance (compound heterozygous)
Mma	NCoR	Possibly linked	Not applicable
	SMRT	Not informative, normal partial sequence	
	NCoA-1	Excluded by sequencing	
	NCoA-3	Possibly linked	
	RXR-γ	Possibly linked	
Mal	NCoR	Excluded	Not applicable
	SMRT	Excluded	
	NCoA-1	Excluded	
	NCoA-3	Excluded	
	RXR-γ	Excluded	
Mch	NCoR	Excluded	Excluded[a]
	SMRT	Excluded	Excluded[a]
	NCoA-1	Excluded by sequencing	Excluded[a]
	NCoA-3	Excluded	Not excluded
	RXR-γ	Possibly linked	Not excluded
Msn	NCoR	Excluded	Not excluded
	SMRT	Excluded	Not informative
	NCoA-1	Excluded	Excluded
	NCoA-3	Excluded[b]	Not excluded
	RXR-γ	Excluded	Excluded

[a] Provided the proposita and her siblings have the same father.
[b] Cannot be excluded if proposita II-1 had a neomutation.

tant TRβ R243Q/W, located in the hinge region of the receptor, binds T_3 poorly when complexed to DNA despite normal T_3 binding in solution (21, 39). As a consequence, dissociation of NCoR and recruitment of NCoA are markedly impaired. Yoh *et al.* (22) reported 11 TRβ mutations with impaired ability to dissociate from corepressors, 2 of which (Δ430M and Δ432G), exhibited unusually strong interaction with SMRT. Moreover, introduction of an amino acid substitution that abolished corepressor association with TR greatly diminished the DNE of the natural TRβ mutants (22, 23). In other instances, impaired interaction of mutant TRs with coactivators played a role in the manifestation of DNE. Natural and artificial mutations in the AF2 region of TRβ showed poor interaction with coactivators (NCoA-1 and RIP140), despite normal or near-normal T_3-binding affinity (25). Nagaya *et al.* (36) demonstrated that an artificial mutation in one of the hydrophobic heptad repeats of the putative receptor dimerization domain (L428R) impaired heterodimerization of TR and RXR. Furthermore, when L428R was introduced in mutant TRβs causing RTH, the DNE of the TRβ mutant was eliminated. From these findings, it can be deduced that abnormal corepressors that fail to dissociate from TRs, defective coactivators that do not associate with TR upon T_3 binding, or abnormal RXR that heterodimerize with TRs but cannot initiate *trans*-activation or have increased affinity for the corepressors could cause dominantly inherited RTH in the absence of TR defects.

Of the two families (Mma and Mal) with dominantly inherited RTH reported herein, we were able to exclude in one (Mal), by sequencing and linkage analysis, the involvement of all four cofactors and RXRγ. In the other family (Mma), the role of NCoA-1 was excluded. Moreover, 85% of the SMRT cDNA-coding sequence was normal. However, the possibility of RTH linkage to NCoR, NCoA-3, and RXRγ could be neither excluded nor proven because of the limited number of family members.

In two families the mode of inheritance could not be determined by pedigree analysis, because only one subject of each family was affected. Assuming autosomal dominant inheritance, the involvement of all four cofactors could be excluded in both families, as well as RXRγ in family Msn. However, if the proposita of family Msn had a neomutation, the involvement of NCoA-3 cannot be excluded. If the inheritance mode were recessive, only NCoA-1 can be excluded in both families. We cannot exclude the involvement of NCoA-3 and RXRγ in family Mch and that of NCoR, SMRT, and NCoA-3 in family Msn.

Although based on pedigree analysis and theoretical considerations, abnormal cofactors or RXRs are likely to cause dominantly inherited RTH, we cannot exclude recessive (compound heterozygous) inheritance in family Msn, given phenotypically normal, nonconsanguineous parents. However, it is equally possible that the occurrence of RTH in subjects with normal parents and siblings represents putative neomutation in a gene, with dominant manifestation. Together with family Mma and 2 other families we previously reported (15, 16), the occurrence of a neomutation in 4 of these 6 families is much higher than the 13% prevalence of the neomutation in RTH caused by TRβ mutations (5, 40). Moreover, there appears to be a reduced penetrance of the RTH phenotype in these families, because only 5 of 22 children born to affected parents are affected. This reduced penetrance, possibly due to reduced survival of embryos, may in part be responsible for the apparently high rate of neomutations.

Given our failure to demonstrate putative defects in 5 proteins intimately involved in the expression of TR-mediated thyroid hormone action, what are the alternative possibilities? Recent studies have uncovered sets of 10–20 mam-

malian proteins that integrate the effects of transcriptional activators of the polymerase II machinery akin to the yeast mediator complex. These protein complexes (SMCC/TRAP, ARC, DRIP, and NAT), isolated by different approaches, are the same or very similar polypeptides (41). Although they interact with a number of nuclear receptors, some, such as TRAP220, interacts directly with TR (42). The constantly increasing number of molecules recognized to be involved in the thyroid hormone-mediated activation of gene transcription has enhanced the complexity of the candidate gene approach in identification of the cause of RTH in subjects without TR gene defects.

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References

1. **Refetoff S, Weiss RE, Usala SJ.** 1993 The syndromes of resistance to thyroid hormone. Endocr Rev. 14:348–399.
2. **Beck-Peccoz P, Chatterjee VK.** 1994 The variable clinical phenotype in thyroid hormone resistance syndrome. Thyroid. 4:225–232.
3. **Refetoff S, DeWind LT, DeGroot LJ.** 1967 Familial syndrome combining deaf-mutism, stuppled epiphyses, goiter and abnormally high PBI: possible target organ refractoriness to thyroid hormone. J Clin Endocrinol Metab. 27:279–294.
4. **Announcement.** 1994 A registry for resistance to thyroid hormone. Mol Endocrinol. 8:1558.
5. **Brucker-Davis F, Skarulis MC, Grace MB, et al.** 1995 Genetic and clinical features of 42 kindreds with resistance to thyroid hormone. The National Institutes of Health Prospective Study. Ann Intern Med. 123:572–583.
6. **Sakurai A, Takeda K, Ain K, et al.** 1989 Generalized resistance to thyroid hormone associated with a mutation in the ligand-binding domain of the human thyroid hormone receptor β. Proc Natl Acad Sci USA. 86:8977–8981.
7. **Usala SJ, Tennyson GE, Bale AE, et al.** 1990 A base mutation of the c-*erb*A β thyroid hormone receptor in a kindred with generalized thyroid hormone resistance. Molecular heterogeneity in two other kindreds. J Clin Invest. 85:93–100.
8. **Beck-Peccoz P, Chatterjee VKK, Chin WW, et al.** 1993 Nomenclature of thyroid hormone receptor β-gene mutations in resistance to thyroid hormone: consensus statement from the first workshop on thyroid hormone resistance, July 10–11, 1993, Cambridge, United Kingdom. J Clin Endocrinol Metab. 78:990–993.
9. **Takeda K, Sakurai A, DeGroot LJ, Refetoff S.** 1992 Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor-β gene. J Clin Endocrinol Metab. 74:49–55.
10. **Takeda K, Balzano S, Sakurai A, DeGroot LJ, Refetoff S.** 1991 Screening of nineteen unrelated families with generalized resistance to thyroid hormone for known point mutations in the thyroid hormone receptor β gene and the detection of a new mutation. J Clin Invest. 87:496–502.
11. **Weiss RE, Weinberg M, Refetoff S.** 1993 Identical mutations in unrelated families with generalized resistance to thyroid hormone occur in cytosine-guanine-rich areas of the thyroid hormone receptor beta gene. Analysis of 15 families. J Clin Invest. 91:2408–2415.
12. **Adams M, Matthews C, Collingwood TN, Tone Y, Beck-Peccoz P, Chatterjee KK.** 1994 Genetic analysis of 29 kindreds with generalized and pituitary resistance to thyroid hormone. Identification of thirteen novel mutations in the thyroid hormone receptor beta gene. J Clin Invest. 94:506–515.
13. **Parrilla R, Mixson AJ, McPherson JA, McClaskey JH, Weintraub BD.** 1991 Characterization of seven novel mutations of the c-*erb*A β gene in unrelated kindreds with generalized thyroid hormone resistance. Evidence for two "hot spot" regions of the ligand binding domain. J Clin Invest. 88:2123–2130.
14. **Collingwood TN, Wagner R, Matthews CH, et al.** 1998 A role for helix 3 of the TRbeta ligand-binding domain in coactivator recruitment identified by characterization of a third cluster of mutations in resistance to thyroid hormone. EMBO J. 17:4760–4770.
15. **Weiss RE, Hayashi Y, Nagaya T, et al.** 1996 Dominant inheritance of resistance to thyroid hormone not linked to defects in the thyroid hormone receptor α or β genes may be due to a defective cofactor. J Clin Endocrinol Metab. 81:4196–4203.
16. **Pohlenz J, Weiss RE, Macchia PE, et al.** 1999 Five new families with resistance to thyroid hormone not caused by mutations in the thyroid hormone receptor beta gene. J Clin Endocrinol Metab. 84:3919–3928.
17. **Refetoff S, Salazar A, Smith TJ, Scherberg NH.** 1983 The consequences of inappropriate treatment because of failure to recognize the syndrome of pituitary and peripheral tissue resistance to thyroid hormone. Metabolism. 32:822–834.
18. **Weiss RE, Balzano S, Scherberg NH, Refetoff S.** 1991 1990 Neonatal detection of generalized resistance to thyroid hormone. JAMA. 264:2245–2250 (published erratum appears in JAMA 2265:2869).
19. **Koenig RJ.** 1998 Thyroid hormone receptor coactivators and corepressors. Thyroid. 8:703–713.
20. **Yen PM, Chin WW.** 1994 Molecular mechanisms of dominant negative activity by nuclear hormone receptors. Mol Endocrinol. 8:1450–1454.
21. **Safer JD, Cohen RN, Hollenberg AN, Wondisford FE.** 1998 Defective release of corepressor by hinge mutants of the thyroid hormone receptor found in patients with resistance to thyroid hormone. J Biol Chem. 273:30175–30182.
22. **Yoh SM, Chatterjee VK, Privalsky ML.** 1997 Thyroid hormone resistance syndrome manifests as an aberrant interaction between mutant T_3 receptors and transcriptional corepressors. Mol Endocrinol. 11:470–480.
23. **Tagami T, Jameson JL.** 1998 Nuclear corepressors enhance the dominant negative activity of mutant receptors that cause resistance to thyroid hormone. Endocrinology. 139:640–650.
24. **Collingwood TN, Rajanayagam O, Adams M, et al.** 1997 A natural transactivation mutation in the thyroid hormone β receptor: impaired interaction with putative transcriptional mediators. Proc Natl Acad Sci USA. 94:248–253.
25. **Liu Y, Takeshita A, Misiti S, Chin WW, Yen PM.** 1998 Lack of coactivator interaction can be a mechanism for dominant negative activity by mutant thyroid hormone receptors. Endocrinology. 139:4197–4204.
26. **Weiss RE, Xu J, Ning G, Pohlenz J, O'Malley BW, Refetoff S.** 1999 Mice deficient in the steroid receptor co-activator 1 (SRC-1) are resistant to thyroid hormone. EMBO J. 18:1900–1904.
27. **Brown NS, Smart A, Sharma V, et al.** 2000 Thyroid hormone resistance and increased metabolic rate in the RXR-γ-deficient mouse. J Clin Invest. 106:73–79.
28. **Bell GI, Karam JH, Rutter WJ.** 1981 Polymorphic DNA region adjacent to the 5′ end of the human insulin gene. Proc Natl Acad Sci USA. 78:5759–6763.
29. **Nagaya T, Chen K-S, Fujieda M, et al.** 1999 Localization of the human nuclear receptor corepressor (hN-CoR) gene between the CMT1A and the SMS critical regions of chromosome 17p11.2. Genomics. 59:339–341.
30. **Hayashi Y, Yamamoto M, Ohmori S, et al.** 1999 Polymorphism of homopolymeric glutamines in coactivators for nuclear hormone receptors. Endocr J. 46:279–284.
31. **Colgan JE, Kruisbeek AM, Margulies DH, Shevaach EM, Strover W,** eds. 1991 Current protocols in immunology. New York: Wiley and Sons; 7.22.
32. **Hayashi Y, Ohmori S, Ito T, Seo H.** 1997 A splicing variant of steroid receptor coactivator-1 (SRC-1E): the major isoform of SRC-1 to mediate thyroid hormone action. Biochem Biophys Res Commun. 236:83–87.
33. **Chen JD, Evans RM.** 1995 A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature. 377:454–457.
34. **Nagaya T, Madison LD, Jameson JL.** 1992 Thyroid hormone receptor mutants that cause resistance to thyroid hormone. Evidence for receptor competition for DNA sequences in target genes. J Biol Chem. 267:13014–13019.
35. **Yen PM, Sugawara A, Refetoff S, Chin WW.** 1992 New insights on the mechanism(s) of the dominant negative effect of mutant thyroid hormone receptor in generalized resistance to thyroid hormone. J Clin Invest. 90:1825–1831.
36. **Nagaya T, Jameson JL.** 1993 Thyroid hormone receptor dimerization is required for dominant negative inhibition by mutations that cause thyroid hormone resistance. J Biol Chem. 268:15766–15771.
37. **Meier CA, Dickstein BM, Ashizawa K, et al.** 1992 Variable transcriptional activity and ligand binding of mutant β1 3,5,3′-triiodothyronine receptors from four families with generalized resistance to thyroid hormone. Mol Endocrinol. 6:248–258.
38. **Hayashi Y, Weiss RE, Sarne DH, et al.** 1995 Do clinical manifestations of resistance to thyroid hormone correlate with the functional alteration of the corresponding mutant thyroid hormone-β receptors? J Clin Endocrinol Metab. 80:3246–3256.
39. **Yagi H, Pohlenz J, Hayashi Y, Sakurai A, Refetoff S.** 1997 Resistance to thyroid hormone caused by two mutant thyroid hormone receptor β, R243Q and R243W, with marked impairment of function that cannot be explained by altered in-vitro 3,5,3′-triiodothyronine binding affinity. J Clin Endocrinol Metab. 82:1608–1614.
40. **Refetoff S, Weiss RE, Usala SJ, Hayashi Y.** 1994 The syndromes of resistance to thyroid hormone: update 1994. In: Braverman LE, Refetoff S, eds. Endocrine reviews monographs. Bethesda: The Endocrine Society; 336–343.
41. **Malik S, Roeder RG.** 2000 Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells. Trends Biochem Sci. 25:277–283.
42. **Ito M, Yuan CX, Malik S, et al.** 1999 Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. Mol Cell. 3:361–370.