CLINICAL STUDY

Genetic analysis of lithium-associated parathyroid tumors

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Abstract

Objective: The aim of this study was to determine the primary genetic events that may underlie the formation of parathyroid tumors in patients with lithium-associated hyperparathyroidism (HPT).

Methods: Comparative genomic hybridization (CGH), loss of heterozygosity (LOH) and multiple endocrine neoplasia type 1 gene (*MEN1*) mutation analysis were used to analyze twelve parathyroid tumors from nine patients with lithium-associated HPT. For comparison, CGH was also carried out in a non-lithium-associated group of thirteen sporadic parathyroid tumors.

Results: A higher prevalence of multiglandular disease in the lithium-associated HPT patients compared with the idiopathic sporadic patients was observed (Fisher's exact test, $P = 0.02$). CGH alterations were detected in four lithium-associated parathyroid tumors, involving loss at 1p, 11, 15q, 22q and gain of the X chromosome. In addition, one of these four cases exhibited LOH at 11q13 and was found to contain a novel somatic *MEN1* mutation (c.1193insTAC). Although fewer lithium-associated parathyroid tumors were shown to contain genetic alterations compared with the sporadic parathyroid tumors, the changes detected were those frequently associated with both familial and sporadic parathyroid tumorigenesis.

Conclusion: This is, to our knowledge, the first genetic analysis of parathyroid tumors in lithium-associated HPT patients. Our data indicated that the majority of lithium-associated parathyroid tumors do not contain gross chromosomal alterations and suggest that in most cases the tumorigenic pathway is independent of *MEN1* and genes at 1p34.3-pter and 1q21-q32. It is possible that other discrete genetic alterations or epigenetic changes, not screened for in this study, could also be responsible for parathyroid tumorigenesis in lithium-associated HPT.

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Introduction

Lithium is widely used in the management of patients with bipolar disorder and has been shown to have adverse effects on a number of tissues, including the parathyroid glands (1, 2). Lithium was first linked to the development of primary hyperparathyroidism (HPT) by Garfinkel *et al.* in 1973 (3) and, since then, numerous reports have shown an association between lithium therapy and HPT (1, 4, 5). The prevalence of HPT developing in patients receiving lithium therapy has been reported to be as high as 15% (6, 7), compared with an estimated prevalence of 1% in the general population (8). The molecular mechanisms underlying the therapeutic actions of lithium have not been fully elucidated; however, it is thought that

signal transduction pathways, such as protein kinase C, guanine nucleotide-binding proteins, adenylyl cyclases and the phosphoinositide cycle are likely targets of lithium's actions (9). Lithium has also been shown to stimulate gene expression through the AP-1 transcription factor pathway (10). To date, no studies have reported analyses of the primary genetic events that may underlie the formation of lithium-associated parathyroid tumors.

Several genetic regions of interest have, however, been associated with both sporadic and familial parathyroid tumorigenesis occurring independently of lithium therapy. Multiple endocrine neoplasia type 1 (*MEN 1*) is an autosomal dominant familial disorder involving mainly the parathyroid glands, enteropancreatic endocrine tissues and the anterior pituitary.

Somatic mutations within *MEN1* have been shown in 7–27% of sporadic parathyroid tumors (11, 12). Germline *MEN1* mutations have been identified in 49–100% of MEN 1 families and in a subset of families with isolated familial hyperparathyroidism (13–15). Another familial disease, hyperparathyroidism-jaw tumor (HPT-JT) syndrome mainly encompasses primary HPT in association with fibro-osseous jaw tumors (16). The HPT-JT gene (*HRPT2*) has been mapped to 1q21-q32 (17) and is thought to be a tumor suppressor gene, based on several studies which have identified allelic loss at the HPT-JT locus in a number of disease-associated tumors (18, 19). Further to this, loss of heterozygosity (LOH) and comparative genomic hybridization (CGH) studies have implicated chromosomal regions frequently altered in familial and/or sporadic parathyroid tumors at 1p, 1q (the HPT-JT locus, *HRPT2*), 6q, 9p, 11q13 (the MEN 1 locus, *MEN1*), 13q, 15q, 17, 19, 22q and the X chromosome (12, 20–27).

Parathyroidectomy in patients with lithium-associated HPT has revealed both multiglandular and uniglandular disease (1, 5). Whilst previously classified as either parathyroid hyperplasia or adenoma, these two entities cannot be reliably histologically differentiated and are more frequently described as either uniglandular (if only one gland is affected) or multiglandular (if more than one gland is affected) (28). It is unclear as to whether lithium initiates disease or promotes underlying HPT or if both mechanisms may be involved. In the case of uniglandular disease, abnormalities usually arise earlier in the course of lithium therapy and it is thought that lithium is likely to promote pre-existing HPT (1). Multiglandular disease, however, is most often associated with longer term lithium therapy and it has been proposed that in these cases lithium may be responsible for initiating HPT (1).

In the present study, we have screened a panel of lithium-associated (three uniglandular and six multiglandular) and non-lithium-associated sporadic (eleven uniglandular and two multiglandular) parathyroid tumors for gross genomic alterations using CGH. In addition, the lithium-associated parathyroid tumors were analyzed for LOH in chromosomes 1 and 11 and screened for *MEN1* mutations.

Subjects and methods

Lithium-associated HPT patients and parathyroid samples

Twelve parathyroid samples were obtained from nine patients (five females and four males) who underwent parathyroidectomy at Royal North Shore Hospital, Sydney, Australia, for lithium-associated primary HPT. Patients were classified as having lithium-associated HPT if, at the time of operation, they were taking lithium and had no family history suggestive of MEN 1

or HPT-JT. Clinical information including lithium dose and duration for each of the patients is summarized in Table 1. Three patients (two females and one male) had uniglandular disease while the other six patients (three females and three males) had multiglandular disease. Serum calcium levels returned to normal post-operatively for all nine patients (Table 1), suggesting that all affected glands were removed at the time of surgery. In those patients with uniglandular disease (LA-1, LA-4, LA-6), the length of lithium treatment ranged from 0.5 to 10 years, while the length of lithium treatment in patients with multiglandular disease (LA-2, LA-3, LA-5, LA-7, LA-8, LA-9) ranged from 12 to greater than 20 years (Table 1). One patient, LA-3, had a parathyroidectomy at 69 years of age, with two affected glands being removed at the time of surgery. The patient became normocalcemic post-operatively and had been on lithium therapy at the time of surgery for more than 17 years. Three years later this patient had two remaining glands removed due to recurrent HPT. A parathyroid sample was only available from the second surgery for genetic analysis in this study. Patients gave informed consent according to a protocol approved by the Royal North Shore Hospital Human Research Ethics Committee.

Sporadic HPT patients and parathyroid samples

Thirteen parathyroid tumors were obtained from thirteen patients (eight females and five males) who underwent parathyroidectomy at the Royal North Shore Hospital for sporadic primary HPT. LOH and *MEN1* mutation analysis, but not CGH, have previously been reported in these tumors (12). Eleven subjects had uniglandular disease, while two had multiglandular disease, both with two affected glands (S-22, S-43). Post-operative serum calcium levels were within the normal range (Table 2). None of the thirteen subjects exhibited any other phenotypic features or family history suggestive of MEN 1 or HPT-JT. The number of glands affected, average gland weight, age at operation, pre- and post-operative serum calcium levels and pre-operative parathyroid hormone (PTH) levels for each of the thirteen sporadic HPT patients are summarized in Table 2. Patients gave informed consent according to a protocol approved by the Royal North Shore Hospital Human Research Ethics Committee.

DNA extraction

Tissue from both the lithium-associated and sporadic parathyroid samples was frozen in liquid nitrogen immediately after surgical removal and stored at –70 °C. Blood samples were obtained from the patients for DNA extraction. DNA was extracted from fresh-frozen tissue samples and peripheral blood leucocytes according to standard procedures (29). At the time

Table 1 Clinical and genetic data for the twelve lithium-associated parathyroid tumors from nine patients.

Patient	Age at operation (years)	Gender	Glands affected	Lithium therapy		Clinical data			Gland analyzed	Gland weight (g)	CGH results		LOH results			MEN1 mutation analysis†	MEN1 polymorphisms
				Duration (years)	Dose (average mg/day)	SCa* (mmol/l)	Post-sCa* (mmol/l)	PTH†			Losses	Gains	1p	1q	11q13		
1	78	F	1	5	750	3.17	2.28	3.55	LA-1	8.06	1pcen-p31, 11q13-qter, 15q	—	+	+	+	—	—
2	52	M	4	16	180	2.97	2.35	2.83	LA-2	0.37	—	—	+	+	+	—	—
3	72	M	4	20+	500	2.81	2.54	3.07	LA-3	0.70	22911.2-q13	X	+	+	+	—	—
4	35	M	1	8–10	750	2.90	2.33	1.90	LA-4	na	11p14-p15, 11q22-q25	—	+	+	LOH	c.1193insTAC	—
5	64	F	2	20	1000	2.73	2.32	1.13	LA-5	0.12	—	—	+	+	+	—	—
6	52	F	1	0.5	500	2.80	2.12	1.68	LA-6	5.63	—	—	+	+	+	—	D418D
7	47	F	2	15+	750	2.93	2.35	4.56	LA-7	0.81	—	—	+	+	+	—	D418D
8	70	M	4	15	500	2.74	2.40	2.91	LA-8a	0.26	—	X	+	+	NI	—	D418D
9	68	F	4	12	750	2.65	2.43	1.07	LA-8b	na	—	—	+	+	NI	—	D418D
									LA-9a	0.08	—	—	+	+	+	—	D418D
									LA-9b	0.06	—	—	+	+	+	—	D418D
									LA-9c	0.03§	—	—	+	+	+	—	D418D

*Pre- and post-operative serum calcium (reference range 2.10–2.60 mmol/l); †pre-operative intact PTH in multiples of the upper normal limit; ‡confirmed somatic mutations; nucleotide numbering based on sequence obtained from EMBL Accession No. U93236; §even though this sample falls below the normal criteria for an abnormal gland (42), histopathologically this sample was found to be hyperplastic. —, none detected; ||, nucleotide change – c.1364 C > T; +, retention of heterozygosity; na, not analysed/not available; NI, not informative (homozygous); LOH, loss of heterozygosity.

Table 2 Clinical and genetic data for the thirteen sporadic parathyroid tumors from thirteen patients.

Patient	Age at operation (years)	Gender	Glands affected	Average affected gland weight (g)	Clinical data			CGH results		LOH results§			MEN1 mutation analysis†§	MEN1 polymorphisms§
					Pre-sCa* (mmol/l)	Post-sCa* (mmol/l)	PTH†	Losses	Gains	1p	1q	11q13		
S-8	63	F	1	0.96	2.99	2.24	3.10	11, 13q	—	+	+	LOH	c.960G > C	—
S-10	17	F	1	3.50	3.04	2.25	5.32	5q14-q31	—	+	+	+	na	—
S-19	62	F	1	0.186	2.77	2.31	0.78	22q	X	+	+	+	na	—
S-22	59	F	2	na	2.66	2.28	4.71	11	—	+	+	LOH	c.1849C > G	—
S-27	49	M	1	3.00	2.82	2.33	6.10	2p	—	LOH	+	+	na	—
S-29	69	F	1	1.70	3.10	2.19	2.87	—	5, 6p, 7, 14q, X	LOH	LOH	LOH	—	c.76T > A
S-31	65	M	1	na	2.75	2.40	0.93	11, 13q	—	+	LOH	LOH	c.707delCinsGG	—
S-33	55	F	1	0.50	2.61	2.44	1.19	1q31-qter	—	LOH	LOH	+	na	—
S-34	42	F	1	1.60	2.89	2.14	5.00	1, 15q	—	LOH	LOH	+	na	—
S-35	64	M	1	3.40	3.18	2.41	na	—	—	+	+	+	na	—
S-38	63	M	1	na	3.05	2.39	1.15	22q	X	+	+	+	na	—
S-41	63	M	1	0.44	2.88	2.29	1.53	—	3	+	+	+	na	—
S-43	76	F	2	0.24	2.62	2.21	1.47	11p11-p15, 11q13-qter, 13q12-q32	—	+	+	LOH	—	R171Q (c.622G > A)

*Pre- and post-operative serum calcium (reference range 2.10–2.60 mmol/l); †pre-operative intact PTH in multiples of the upper normal limit; ‡confirmed somatic mutations; nucleotide numbering based on sequence obtained from EMBL Accession No. U93236; §previously reported (12). —, none detected; +, retention of heterozygosity; na, not analysed/not available; LOH, loss of heterozygosity.

of extraction, a representative piece of tissue was formalin-fixed and paraffin-embedded and tumor status was confirmed histologically. All lithium-associated and sporadic samples were histologically shown to be composed of at least 80% tumor cells or hyperplasia, with the exception of two samples, LA-8a and S-31, which were composed of 50% and 60% tumor cells respectively.

CGH studies

Genomic DNA from twelve parathyroid lesions from nine patients with lithium-associated HPT and thirteen parathyroid tumors from thirteen patients with sporadic primary HPT were analyzed by CGH as previously described (26). Briefly, tumor DNA samples were labeled with fluorescein-12-dUTP (NEN, Life Science Products, Boston, MA, USA) by nick translation and normal reference DNA was labeled with SpectrumRed (Vysis, Inc., Downers Grove, IL, USA). Tumor and reference DNA were mixed with unlabeled Cot-1 DNA (Life Technologies, Inc., Gaithersburg, MD, USA), denatured and hybridized to normal male human metaphase chromosomes (Vysis, Inc.). After hybridization at 37°C for 72 h, the slides were washed in $0.4 \times \text{SSC}/0.3\%$ Nonidet P-40 (Boehringer Mannheim GmbH, Mannheim, Germany) at 74°C for 2 min, $2 \times \text{SSC}/0.1\%$ Nonidet P-40 at room temperature for 2 min, followed by water at room temperature for 2 min before being air dried. The chromosomes were counterstained with 4,6-diamidino-2-phenylindole (Vysis, Inc.) to enable identification. For each hybridization, a control experiment was performed to ensure adequate hybridization conditions, in which normal male genomic DNA (Promega, Madison, WI, USA) and normal female genomic DNA (Vysis, Inc.) were hybridized against normal metaphases. A minimum of ten metaphases was captured for each analysis. Green to red ratios of greater than 1.20 were considered as gains of genetic material, and ratios less than 0.80 were considered as losses.

LOH studies

Allelic deletions of chromosome 1 were assessed using the following microsatellite markers (1pter-1qter): D1S243, D1S468, D1S244, D1S2667, D1S228, D1S2728, D1S507, D1S478, D1S513 and MYCL in 1p; and D1S218, D1S215, D1S191, D1S222, D1S428, D1S412, D1S413, D1S477 and D1S423 in 1q (12, 30) (<http://www.genome.wi.mit.edu>; <http://gdbwww.gdb.org>; <http://www.ncbi.nlm.nih.gov/genemap99>). LOH at the MEN1 locus, 11q13, was analyzed using PYGM, D11S4946 (within MEN1), D11S4940 and D11S449 (12, 31).

Polymerase chain reactions (PCR) were used to amplify genomic DNA (50 ng) from blood and tumor samples as previously described (12). LOH was deter-

mined visually and quantitatively by comparison of intensities of the two alleles in informative cases. LOH was confirmed if a reduction of >50% of the signal intensity in the tumor sample allele compared with that of the constitutional DNA was observed, as previously described for this method (12, 22).

MEN1 mutation analysis

Mutation analysis was performed by direct sequencing of the nine coding exons of MEN1. Tumor DNA (100 ng) was amplified using 14 different fragments of 200–300 bp each and standard PCR conditions, as previously described (12). The PCR product was then purified using the Wizard PCR Preps DNA Purification System (Promega) and underwent cycle sequencing using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Perkin-Elmer Corporation, Foster City, CA, USA) with thermocycling conditions as follows: 94°C for 5 min; followed by 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. The product was then electrophoresed on 4.8% PAGE Plus gel (Amresco, Cleveland, OH, USA) and analyzed on a 377XL automated DNA sequencer (Applied Biosystems, Perkin-Elmer Corporation).

Statistical analysis of data

Statistical analysis was carried out in order to determine whether the prevalence of multiglandular disease occurring in the lithium-associated HPT patients was higher than that observed in the group of idiopathic sporadic HPT patients, using Fisher's exact test (Stat-View software; Abacus Concepts, Inc., Berkeley, CA, USA).

Results

Prevalence of multiglandular disease

The prevalence of multiglandular disease occurring in the lithium-associated HPT patients (six of a total of nine patients) was shown to be significantly higher than that observed in the group of idiopathic sporadic HPT patients (two of a total of thirteen patients), using Fisher's exact test ($P = 0.02$).

CGH alterations in lithium-associated parathyroid tumors

Twelve parathyroid lesions from nine lithium-associated HPT patients were analyzed by CGH. The gains and losses found are detailed in Table 1, together with the clinical information. Subchromosomal regions with increased and decreased DNA sequence copy number changes are illustrated in Fig. 1a. Alterations, involving 1–3 regions, were detected in four of the twelve

samples (33%). Of those four tumors showing DNA sequence copy number changes, there was an average of two genetic alterations per sample. Two uniglandular samples (LA-1 and LA-4) both exhibited loss at 11q, but did not exhibit any gains. The loss detected by CGH at 11q in LA-1 extended only partially into the 11q13 sub-band where *MEN1* is located. Loss was also detected at 1p and 15q in LA-1, while loss of 11p was also seen in LA-4. One of the uniglandular samples (LA-6) failed to exhibit any change detectable by CGH. Two multiglandular samples from different patients (LA-3 and LA-8a) showed chromosomal imbalances by CGH, with one of these samples (LA-3) exhibiting loss of 22q and gain of the X chromosome. It is interesting to note that although gain of the X chromosome was seen in LA-8a, the paired parathyroid gland (LA-8b) did not exhibit any gains or losses detectable by CGH. The remaining six multiglandular

samples (LA-2, LA-5, LA-7, LA-9a, LA-9b and LA-9c) did not exhibit any alterations detectable by CGH.

CGH alterations in the sporadic parathyroid tumors

Thirteen parathyroid lesions from thirteen sporadic primary HPT patients were analyzed by CGH. The gains and losses found are detailed in Table 2, together with the clinical information. Further to this, sub-chromosomal regions with increased and decreased DNA sequence copy number changes are illustrated in Fig. 1b. Twelve of the thirteen (92%) parathyroid lesions were found to exhibit alterations, involving 1–5 regions, with an average of 1.9 changes per sample in those tumors exhibiting changes. Loss at chromosome 11 was the most frequently detected change in the sporadic parathyroid tumors, with four of the thirteen (31%) tumors exhibiting partial or total loss of the chromosome. Loss of 13q was detected in three of the thirteen (23%) tumors, while gain across the entire X chromosome was seen in three of thirteen sporadic parathyroid tumors. Two of thirteen (15%) tumors exhibited loss at 1q, while loss in two of the thirteen tumors was also detected at 22q. Loss at 2p, 5q14–q31 and 15q was detected only once, while gains of 3, 5, 6p, 7 and 14q were also detected once. Although LOH was observed at 11q13 in the parathyroid tumor from patient S-29 (12), no loss in this sample was shown by CGH analysis (Table 2). The lower resolution of CGH could explain this result, as the region of loss detected by LOH may be too small to be detected by CGH.

Figure 1 Summary of DNA copy number changes detected by CGH in the (a) twelve lithium-associated parathyroid tumors (number of tumors in which changes were detected (n) = 4) and (b) thirteen idiopathic sporadic parathyroid tumors (number of tumors in which changes were detected (n) = 12) analyzed. Each line represents one alteration detected in one tumor, with losses illustrated to the left and gains to the right of the ideograms. Those chromosomes which are not illustrated did not exhibit DNA copy number changes, as detected by CGH.

LOH analyses in lithium-associated parathyroid tumors

LOH analyses of chromosomes 1 and 11 were performed on twelve parathyroid samples from nine patients with lithium-associated HPT. The results for these cases are summarized in Table 1. Sample LA-4 was the only sample to show allelic loss at any of the loci analyzed. In this sample, LOH was detected at 11q13 for all informative markers (Fig. 2a). No further loss was detected in sample LA-4 at any of the informative loci in chromosome 1 (Fig. 2a). The two parathyroid lesions from patient LA-8 were not informative at any of the loci analyzed at 11q13. To exclude the possibility of gross deletion at 11q13 in this sample we assessed a heterozygous polymorphism, D418D (c.1364C > T) (32) within *MEN1* in blood and tumor samples from patient LA-8. Both the blood and tumor samples were found to be heterozygous for the polymorphism (D418D), suggesting that, at this particular site, no gross chromosomal deletion had occurred. Although loss of 11q was detected by CGH in LA-1, this loss extended only partially into the 11q13 sub-band where *MEN1* is located and it is possible that

MEN1 lies centromeric of the region detected by CGH. This may explain the retention of heterozygosity observed at all informative markers at and surrounding *MEN1* in this sample. The loss detected by CGH in LA-1 extended from 1cen-p31 and would therefore be consistent with the retention of heterozygosity, spanning 1p34.3-pter, observed in the LOH analysis.

***MEN1* mutation analysis in lithium-associated parathyroid tumors**

MEN1 was sequenced in twelve parathyroid samples from the nine patients with lithium-associated HPT (Table 1). Sequencing of the remaining allele in the tumor DNA from patient LA-4 (Fig. 2b) revealed an insertion of 3 nucleotides in exon 8 (c.1193insTAC). Wild-type sequence was present in the constitutional DNA of patient LA-4 (Fig. 2b), thus confirming the mutation as somatic. No *MEN1* mutations were detected in any of the parathyroid samples from the eight remaining patients; however, seven parathyroid samples and corresponding blood samples (LA-6, LA-7, LA-8a, LA-8b, LA-9a, LA-9b, LA-9c) from four patients were found to contain the polymorphism, D418D, in exon 9.

Discussion

Numerous studies have investigated the pathogenesis of both familial parathyroid tumors (13, 19, 22, 25) and sporadic parathyroid tumors (12, 20–24, 26, 27). However, the genetic changes associated with the development of lithium-associated parathyroid tumors have not previously been reported. In this study, chromosomal changes were identified in both the lithium-associated and sporadic parathyroid tumors;

however, there were fewer changes detected in the lithium-associated parathyroid group. LOH was detected at 11q13 in a single lithium-associated tumor, with a somatic *MEN1* mutation identified on the remaining allele. No additional LOH was detected at any of the three regions analyzed (1p, 1q and 11q13), nor were there any additional somatic *MEN1* mutations detected in the lithium-associated parathyroid tumors. Taken together, these findings suggest that gross chromosomal alterations occur infrequently in lithium-associated parathyroid tumors and, more specifically, the tumorigenic pathway in most cases would appear to be independent of *MEN1* and genes at 1p34.3-pter and 1q21-q32. However, it is possible that epigenetic changes or more discrete genetic alterations, other than those occurring at 1p34.3-pter, 1q21-q32 and 11q13, are responsible for parathyroid tumorigenesis in lithium-associated HPT.

DNA sequence copy number changes, detected by CGH analyses, have been demonstrated at most chromosomal regions in benign sporadic parathyroid tumors (23, 24, 26). CGH analyses in the lithium-associated parathyroid tumors were unable to identify novel regions of gain or loss and did not detect specific genetic changes occurring at high frequency. Although fewer lithium-associated parathyroid tumors (33%) were shown to contain genetic alterations compared with the sporadic parathyroid tumors (92%), the changes detected were those frequently associated with both familial and sporadic parathyroid tumorigenesis. Our ability to readily detect CGH changes in four out of the twelve parathyroid tumors supports previous studies showing the monoclonal nature of parathyroid tumors (33). The low frequency of CGH changes detected in the lithium-associated parathyroid tumors may be due to balanced alterations, such as translocations, inversions or loss of one allele with

Figure 2 (a) Representative phosphorimages from LA-4 demonstrating LOH at 11q13 and retention of heterozygosity at 1p and 1q. At each represented locus, the DNA from the leucocytes (N) were heterozygous and DNA from the tumor (T) cells either retained heterozygosity or lost an allele. Part (i) demonstrates retention of heterozygosity at 1p with D1S513, part (ii) demonstrates retention of heterozygosity at 1q with D1S215 and part (iii) demonstrates LOH at 11q13 with D11S4940 (loss of the upper allele). (b) Automated sequencing of the tumor DNA from LA-4 revealed an insertion of three nucleotides in exon 8 of the *MEN1* gene (c.1193insTAC), while the normal nucleotide sequence was found in the constitutional DNA.

duplication of the other, which are not detected by CGH. Another explanation is that there may be more discrete alterations that are beyond the resolution capabilities of CGH. It is of interest to note that there was a predominance of chromosomal losses, when compared with gains, observed by CGH analyses in both the sporadic and lithium-associated parathyroid tumors. This may suggest that more tumor suppressor genes are involved in the development of these parathyroid tumors, as opposed to oncogenes. However, it is also possible that oncogenes may be involved at a similar frequency, by mechanisms such as translocations or point mutations, which are not detectable by CGH methodology.

LOH analysis of regions at 1p, 1q and 11q13 were performed to determine whether these regions are involved in lithium-associated parathyroid tumorigenesis. LOH at distal 1p, 1q21-q32 (linked to HPT-JT) and 11q13 (the *MEN1* locus) has been demonstrated in both familial and sporadic parathyroid tumors (12, 20–22, 25, 27, 34–36). In this study, LOH analyses of two regions on chromosome 1 at 1p34.3-pter and 1q21-qter (encompassing the HPT-JT locus at 1q21-q32) failed to show allelic loss at any of the informative loci in the twelve lithium-associated parathyroid tumors. Based on previous studies of sporadic parathyroid tumors in which loss at 1p and 1q21-q32 has been shown in 0–44% and 9–16% of samples respectively (12, 20, 22, 37), loss at 1p and 1q may have been expected in at least a proportion of the lithium-associated parathyroid tumors. Our data at chromosome 1 could be interpreted in one of two ways. First, it is possible, but unlikely, that LOH at 1p and 1q was not detected in our study due to relatively small sample size. Alternatively, a more likely explanation is that in lithium-associated parathyroid tumorigenesis, loss of a putative tumor suppressor gene(s) at distal 1p (1p34.3-pter) and 1q21-q32 (encompassing the HPT-JT locus) is not critical for tumor development.

In the present study, LOH at 11q13 was detected in one of eight (12.5%) informative lithium-associated parathyroid tumors. This parathyroid tumor was also shown to exhibit loss at 11q by CGH analysis and was shown to contain a likely novel somatic *MEN1*-mutation (c.1193insTAC). The mutation effectively inserts another amino acid (tyrosine) and is likely therefore to affect the tertiary structure of the menin protein. In addition, in comparison with the mouse (38) and rat (39) *MEN1* sequences show that this mutation occurs in a conserved region and thus is likely to be of functional significance. The incidence of somatic *MEN1* mutations arising in this group of lithium-associated parathyroid tumors fits within the range previously reported in sporadic parathyroid tumors (7–27%) (11, 12). These findings suggest that it is likely that the use of lithium, at therapeutic levels, does not increase the risk of developing a parathyroid tumor(s) as a result of *MEN1* involvement.

It has previously been proposed that lithium may promote pre-existing primary HPT and thus result in uniglandular disease, whereas multiglandular disease may be initiated by lithium (1). The finding of increased prevalence of multiglandular parathyroid disease in this group of lithium-associated HPT patients, when compared with that occurring in idiopathic sporadic HPT patients, is supportive of the hypothesis that lithium may initiate multiglandular HPT. However, analysis of a larger cohort of lithium-associated HPT patients is required to confirm these findings. The anecdotal finding of a higher ratio of male to female patients (4:5) with lithium-associated HPT in this study, compared with that seen in idiopathic sporadic HPT, is also supportive of a difference in the etiology of lithium-associated HPT compared with other sporadic HPT. This is unlikely to be due to an uneven ratio of patients being treated with lithium since the sex ratio in patients with bipolar affective disorders is approximately equal (40).

The hypothesis can be proposed that lithium acts to increase parathyroid cell proliferation which increases the probability of a subsequent genetic event. An increase in parathyroid cell proliferation is supported by evidence of a direct effect of lithium on calcium-regulated PTH secretion by parathyroid cells *in vitro* (41). The physiological or pharmacological stimulus of lithium on proliferation could then predispose the cells to a later genetic event. In different glands within the same patient, these later events could differ, as noted in one of the multiglandular lithium-associated HPT patients (LA-8), where gain of the X chromosome was detected by CGH in one gland, but no CGH changes were detected in the other gland.

In conclusion, the results of this study indicate that the genetic events in lithium-associated HPT, which may occur subsequent to an effect of lithium on increased parathyroid cell proliferation, are unlikely to include gross chromosomal gains or losses. They may involve other discrete chromosomal regions not analyzed in this study, which has indicated that, in most cases, the tumorigenic pathway is independent of *MEN1* and genes at 1p34.3-pter and 1q21-q32.

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