Molecular analysis of the HPJ-JT syndrome and sporadic parathyroid carcinogenesis

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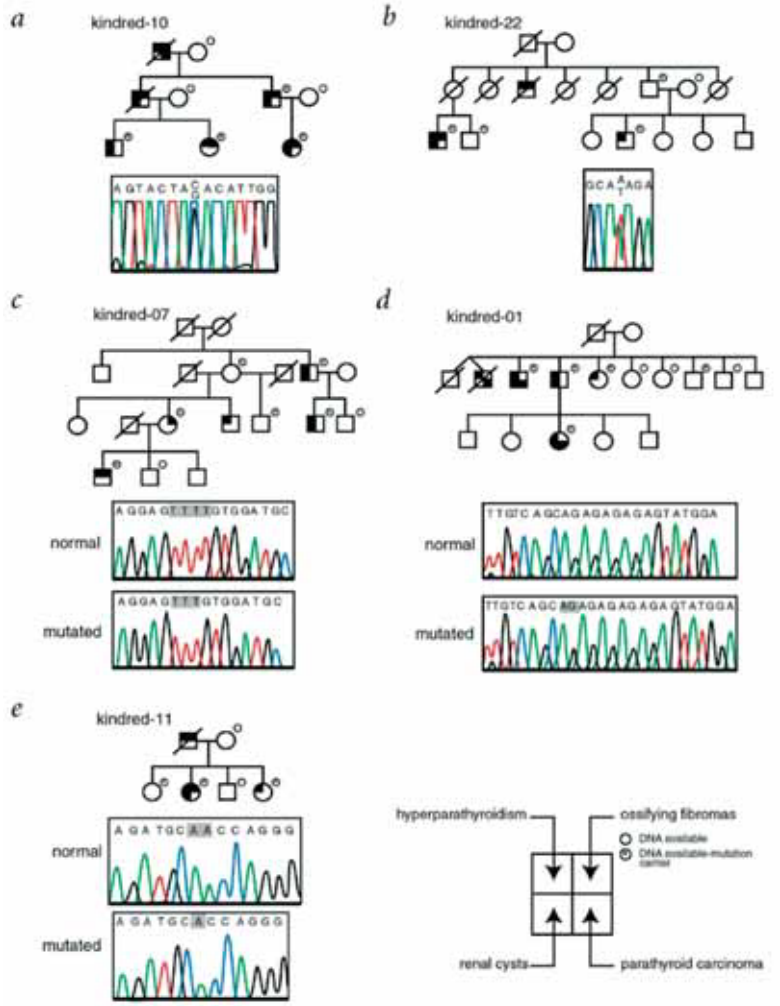
Colour Figure Overview

FIGURE 3.2 Mutations in kindreds affected with HPT-JT.

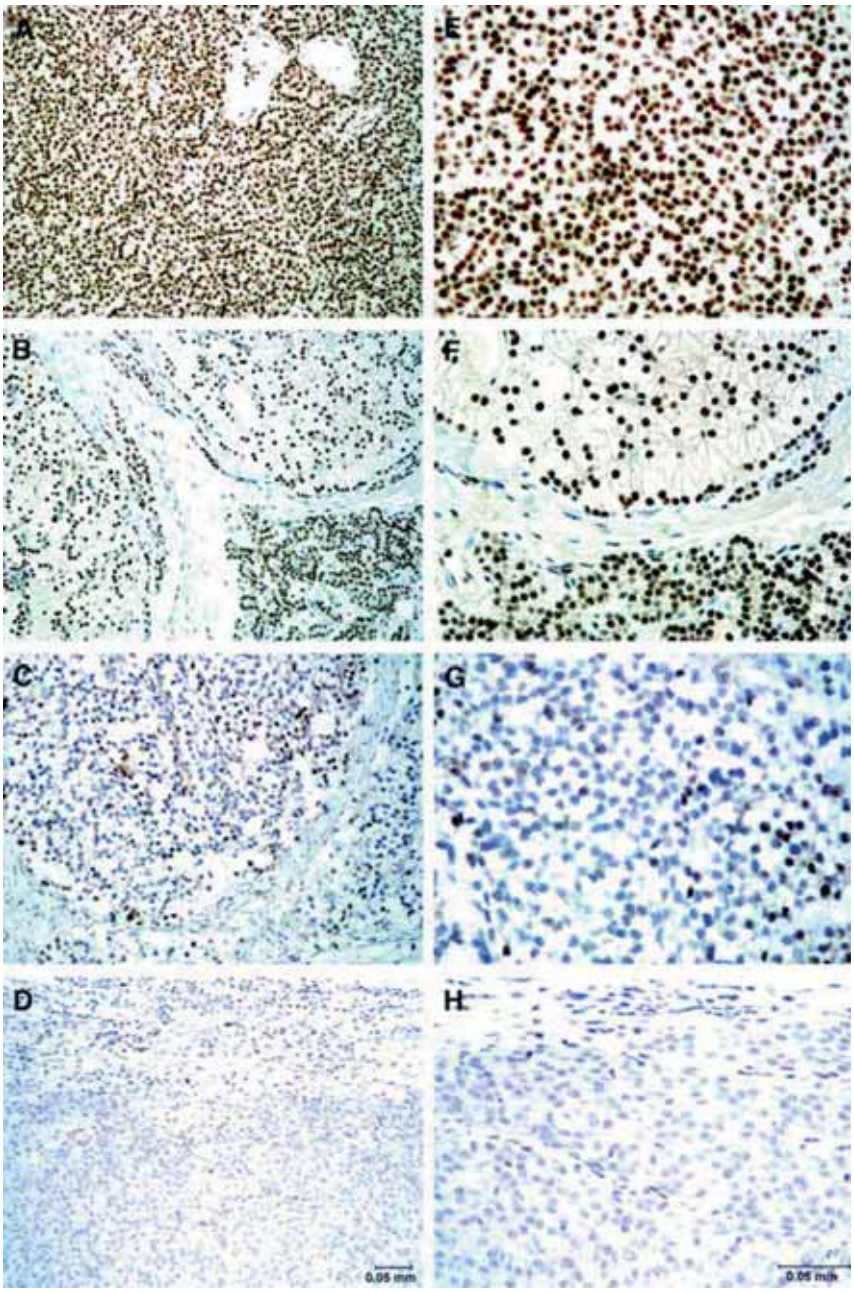


Shaded upper left quadrant represents hyperparathyroidism, upper right quadrant represents ossifying fibroma of the jaw, lower left quadrant represents renal cysts or other kidney tumors, and lower right quadrant represents parathyroid carcinoma. A line drawn through a symbol represents a deceased individual. Completely open symbols represent individuals who are currently unaffected. Small superscript circles to the upper right of family member symbols represent those individuals for whom DNA was available for mutational analysis. Small superscript circles with an asterisk (*) in the middle represent those individuals who are confirmed mutation carriers. a, Kindred-10 and chromatogram showing the heterozygous 165CG nonsense mutation in exon 2. b, Kindred-22 and chromatogram showing the heterozygous 406AT nonsense mutation in exon 5. c, Kindred-07 and chromatograms showing the normal allele and corresponding 636delT mutated allele in exon 7. d, Kindred-01 and chromatograms showing the normal allele and corresponding 679insAG mutated allele in exon 7. e, Kindred-11 and chromatograms showing the normal allele and corresponding 1238delA mutated allele in exon 14. For c-e, PCR products from single affected individuals carrying mutations were subcloned and subsequently sequenced to obtain sequences for both the mutated and normal alleles from the same individual.

FIGURE 5.1 Confocal images demonstrating co-localization of the GFP-parafibromin fusion protein with anti-parafibromin antibody within the nuclei of transfected HEK293 cells.

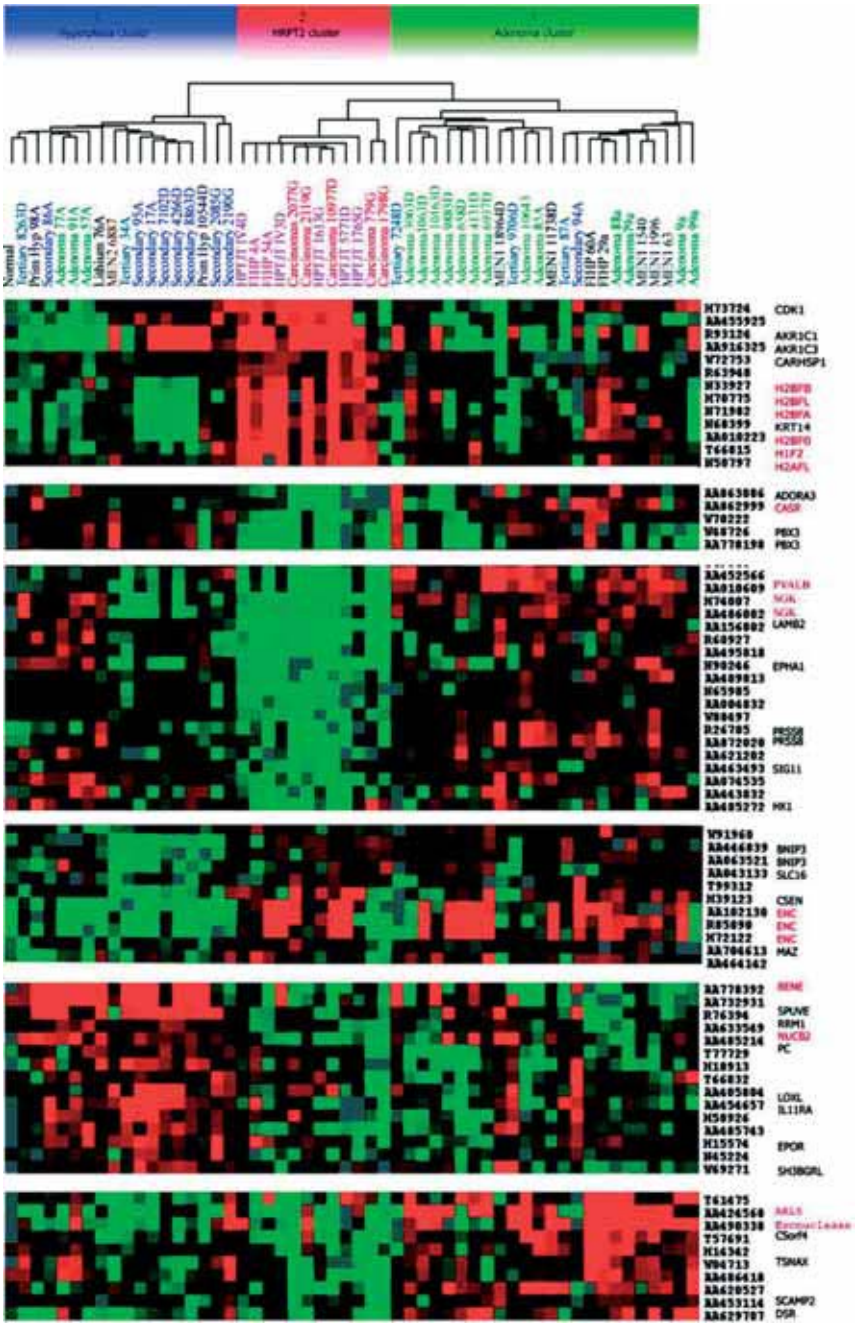
From left, GFP-parafibromin fusion protein expression (green); anti-parafibromin monoclonal antibody binding, as detected by secondary Rhodamine-Red goat antimouse antibody (red); Nomarski image of cells; 4',6-diamidino-2-phenylindole staining of nuclei (blue); superimposition of all images demonstrating co-localization within nuclei. All images captured with a Zeiss LSM510 META laser-scanning confocal microscope.

FIGURE 5.3 Immunohistochemical staining representing the various staining patterns manifested in the different pathologies through parafibromin immunostaining



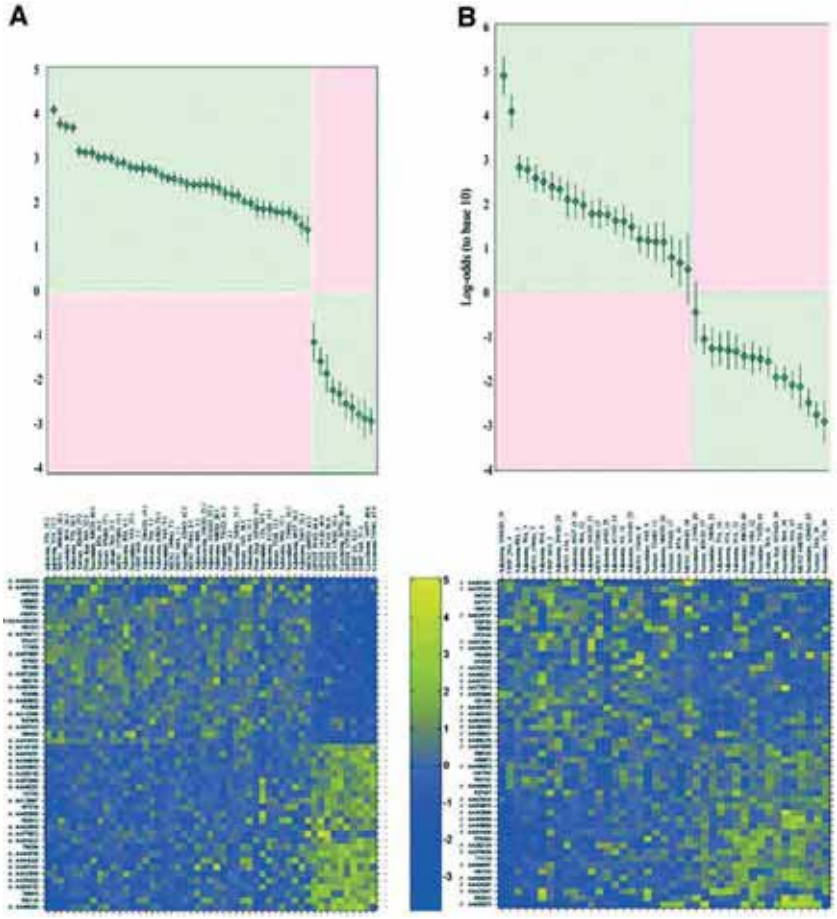
A-D, magnification, 200x. A, diffuse staining (primary parathyroid hyperplasia); B, diffuse staining (sporadic adenoma with a rim of normal tissue); C, focal loss (parathyroid carcinoma); and D, diffuse loss (parathyroid carcinoma). E-H, higher magnifications of the respective parathyroid pathologies at a magnification of 400x. All images were taken with a Spot Insight Camera on a Nikon Eclipse E600.

FIGURE 8.1 Detailed sample dendrogram of unsupervised hierarchical clustering.



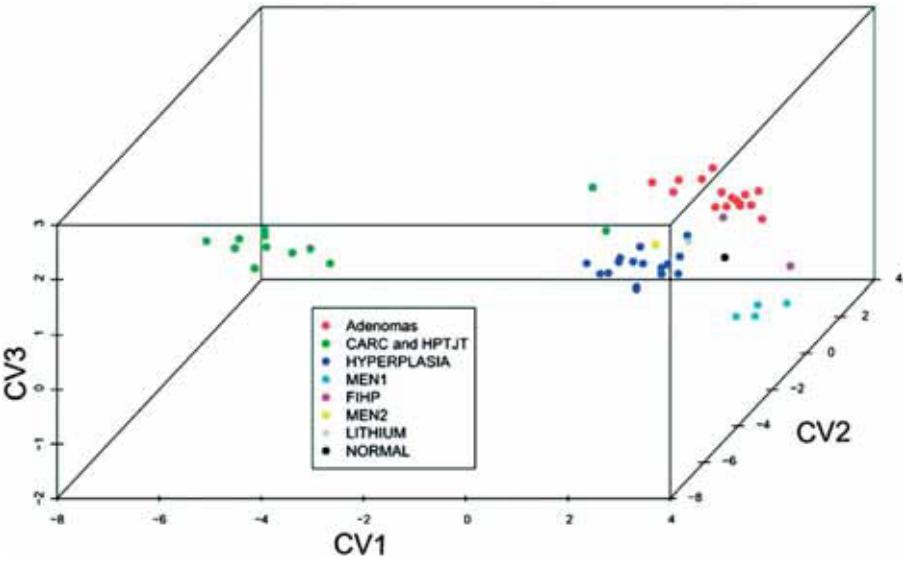
Red and green indicate transcript expression levels respectively above and below the median (black) for each gene across all samples. Grey squares indicate no results.

FIGURE 8.3 Results of classification with a penalized logistic regression model, based on two sets of 50 most significant genes (according to Significance Analysis of Microarray) present in all arrays.



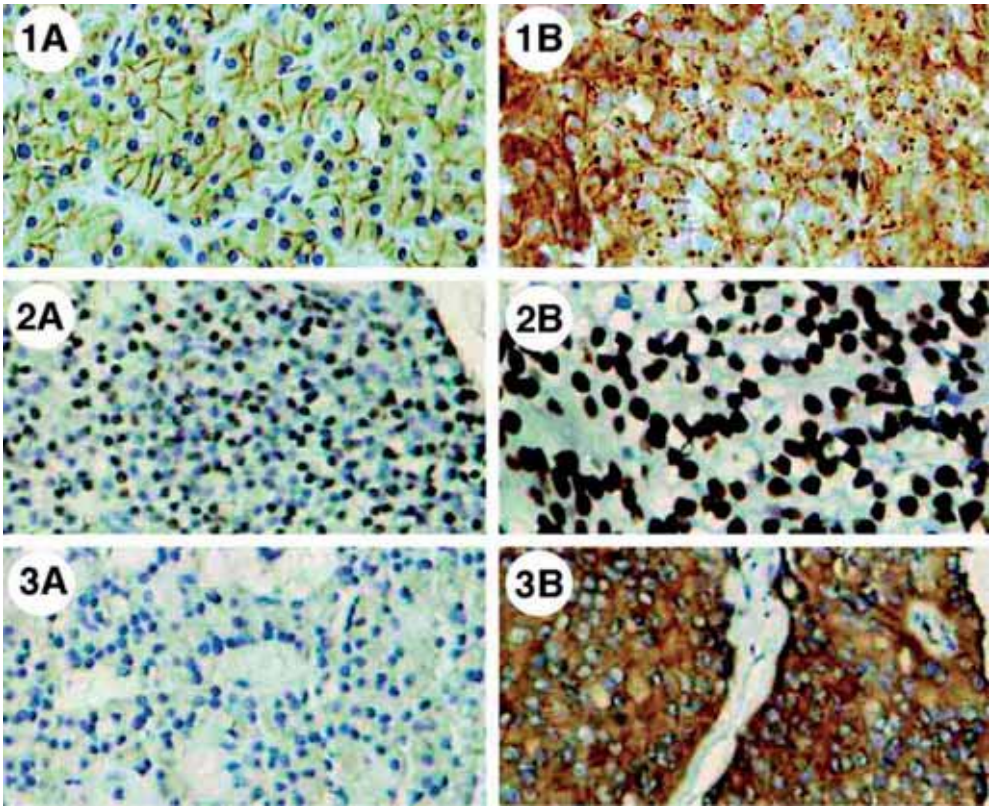
Top panel depicts the results of 100 bootstrap replications: means ([circle]) ±1 SD (lines) of log-odds per array. The vertical scale is log-odds to base 10, where "0" represents equal odds, so that in A, "2" indicates a probability of 100 to 1 that the specimen is not a cluster 2 tumor, and "–3" alternately indicates a probability of 1000 to 1 that the specimen is a cluster 2 tumor. Outcomes in the green (pink) regions are correctly (incorrectly) classified. A represents step-1 differentiation between non-Hyperparathyroidism-Jaw Tumor Syndrome/carcinoma tumors (clusters 1 and 3) and Hyperparathyroidism-Jaw Tumor Syndrome/carcinoma tumors (cluster 2) using 50 genes. B represents step-2 differentiation between adenomas (cluster 3) and the remainder (cluster 1) using a different set of 50 genes. Bottom panel provides graphical presentation of the log expressions with the arrays in the same order as in the top panel. The genes have been ordered along the values of their coefficients in the model with yellow squares indicating the highest expression.

FIGURE 8.4 Canonical variate plot of all arrays.



The plot shows the first three canonical variates (CV1–3). CV1 consisted of 125 genes, CV2, 57 genes, and CV3, 63 genes. Among genes of significance in CV1–3 were CDH1, APP, UCHL1, IGSF4, MOX2, and GAD1. A large separation between the carcinoma/Hyperparathyroidism-Jaw Tumor Syndrome group and the rest of the tumors is evident. The 2 familial isolated hyperparathyroidism specimens with HRPT2 mutations are included in the carcinoma and Hyperparathyroidism-Jaw Tumor Syndrome group that is depicted in green. The 2 (green) carcinomas distant from the main Hyperparathyroidism-Jaw Tumor Syndrome/carcinoma cluster are the outliers #779G and #1798G. Clear separation of the adenoma (red), hyperplasia (purple), and MEN 1 (light blue) groups is also evident. Two MEN 1 tumors are overlaid in this analysis and appear as one blue spot. The pooled normal (black) is among between the adenoma, hyperplasia, and MEN 1 groups. The 2 familial isolated hyperparathyroidism tumors with linkage at 11q13 (pink) are located between the adenomas and MEN 1 tumors. The MEN 2A (yellow) and lithium-associated tumor (gray) are situated closest to the hyperplasia group.

FIGURE 8.5 Immunohistochemical staining



Differential immunohistochemical staining at x200 magnification between (A) adenomas and (B) carcinomas (overexpression) for E-cadherin (1), histone H1 (2), and amyloid ßA4 precursor protein (3).

Curriculum Vitae

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1983-1989	VWO Rijks Scholen Gemeenschap, Heerenveen
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