# Novel *GREM1* enhancer region duplications and associated phenotypes

Raymond C. Chan*, Jeroen Van den Akker*, Zheng Tan, Natalie Lang, Robert O'Conner, Serra Kim, Gilad Mishne, Anjali Zimmer, Annette Leon, Jack Ji (*co-first authors)
Color Genomics, Burlingame, CA

## Introduction

Hereditary mixed polyposis syndrome (HMPS) is an autosomal dominant inherited cancer syndrome characterized by colorectal polyps of multiple or overlapping histological types. HMPS is caused by duplications of the upstream regulatory region of the Gremlin 1 *(GREM1*) gene that include the 3′ region of the adjacent *SCG5* gene. A duplication of 40kb was originally described in HMPS families of Ashkenazi Jewish descent[1]. Subsequently, duplications of 16 kb[2] and 24kb[3] that centered around the predicted transcriptional enhancer of *GREM1* have also been associated with polyposis in a Swedish atypical polyposis family[2] and a non-Ashkenazi Jewish proband with 20 colon polyps of two histological types[3], respectively. In contrast, a larger 57kb duplication that included the upstream regulatory region and the entire coding region of *GREM1* was found associated with colorectal cancer, however, polyposis was absent[4].

Here we report on 14 individuals who have a heterozygous duplication or triplication that encompassed the putative enhancer region or the entirety of the *GREM1* gene. In addition to the two previously reported *GREM1* duplications described above, we present six novel duplications and one novel triplication.

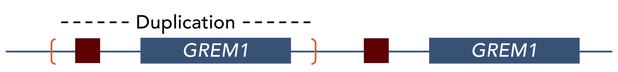
### Current model

Wild-type
Expected phenotype: normal


Enhancer duplication
Expected phenotypes: Colorectal cancer & polyps


### Unresolved

Whole-gene duplication




## Methods

All individuals were ordered the Color Hereditary Cancer Test by a healthcare provider which analyzes 30 genes associated with hereditary cancer. Copy number gain in *GREM1* including the upstream regulatory region was detected by structural variant detection algorithms and were confirmed by orthogonal technologies such as PCR and Sanger sequencing and/or multiplex ligation-dependent probe amplification assay. Laboratory procedures and bioinformatics analysis were performed at the Color laboratory under CLIA and CAP compliance. Variants were classified according to the American College of Medical Genetics and Genomics 2015 guidelines for sequence variant interpretation, and all variant classifications were signed out by a board certified medical geneticist or pathologist. All information was reported by the individual; information not provided was noted as such.

### References

1. Jaeger, E. *et al. Nat. Genet.* 2012.
2. Rohlin, A. *et al. Genes Chromosomes Cancer.* 2016.
3. McKenna, D. B. *Fam. Cancer.* 2018.
4. Venkatachalam, R. *et al. Int. J. Cancer.* 2011.
5. Laitman, *et al. Genet. Res.* 2015.
6. Lieberman, S. *et al. Gastroenterology.* 2017.
7. Davis, H. *et al. Nat. Med.* 2015.

## Results

**Figure 1. Genomic region encompassing *GREM1* and *SCG5*.**

At the gene level, the exons and introns are represented by boxes and the lines with arrows, respectively. The position of the putative *GREM1* enhancer is indicated by the red box. The size of the duplication (kb or Mb) and the number of probands (n) are indicated in each bar. The first four duplications from the top have been confirmed by PCR and Sanger sequencing, and the breakpoints have been resolved. The last five duplications have been confirmed by MLPA, using probes that spanned the 3′ end of *SCG5* and the whole *GREM1* gene.

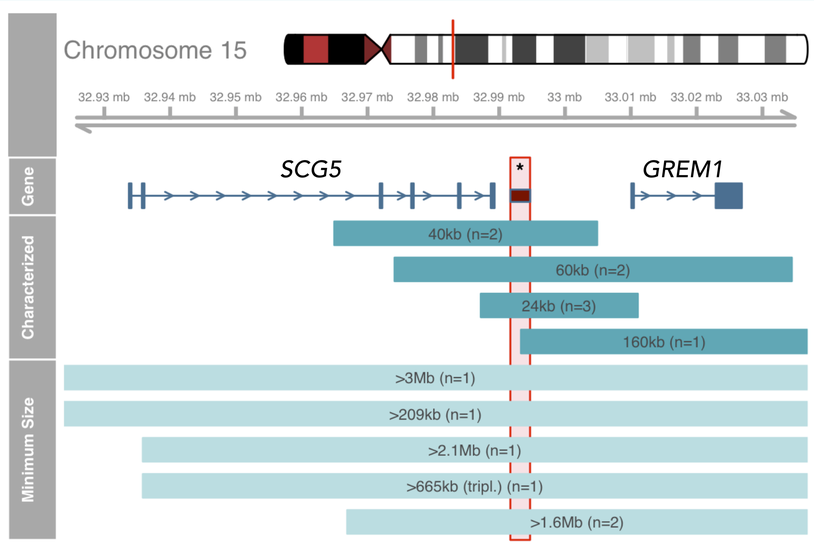



**Table 1. Summary of health history for *GREM1* duplication and triplication carriers.**

Consistent with published reports[1–3,5,6], the 40kb and 24kb duplications of the enhancer region are strongly associated with personal history of colon polyps and family history of colorectal cancer.

| *GREM1* Included Regions | CN Gain Label | Proband | Personal health history | | | | Family health history | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | CRC | Colon polyps | Other cancer | | CRC | Colon polyps | Other cancer | |
| | | | | | age <50 | age ≥50 | | | age <50 | age ≥50 |
| Enhancer | 40kb | 1 | | ✓ | | | ✓ | | | ✓ |
| | | 1.1 | | ✓ | | | | | | |
| | 24kb | 2 | | ✓ | | | ✓ | ✓ | | |
| | | 3 | | ✓ | | | ✓ | | ✓ | |
| | | 4 | no health history provided | | | | | | | |
| Enhancer + whole coding region | 60kb | 5 | | ✓ | | | ✓ | ✓ | | ✓ |
| | | 6 | | | | | | | ✓ | ✓ |
| | 160kb | 7 | no health history provided | | | | | | | |
| | >1.6Mb | 8 | ✓ | | | | ✓ | | ✓ | ✓ |
| | | 8.1 | | | | | | | | |
| | >3Mb | 9 | | | | | | | | |
| | >209kb | 10 | | | ✓ | | | | ✓ | ✓ |
| | >2.1Mb | 11 | no health history provided | | | | | | | |
| | >650kb (triplication) | 12 | | ✓ | | | | | | ✓ |

### A 24kb *GREM1* enhancer duplication

- We have previously described the duplication in Proband 2, a non-Ashkenazi Jewish individual suspected of HMPS[3]. However, this individual also carries a pathogenic *BRCA1* mutation, c.4117G>T (p.E1373*), confounding the association between the *GREM1* duplication and the cancer and hyperplasia clinical phenotypes.
- We identified the same duplication in Probands 3 and 4. These individuals did not have any other pathogenic variants in the 30-gene panel.
  - Notably, Proband 3 has had multiple polyps removed during several colonoscopies, as well as a family history of colorectal and uterine cancer.

### *GREM1* whole-gene duplications

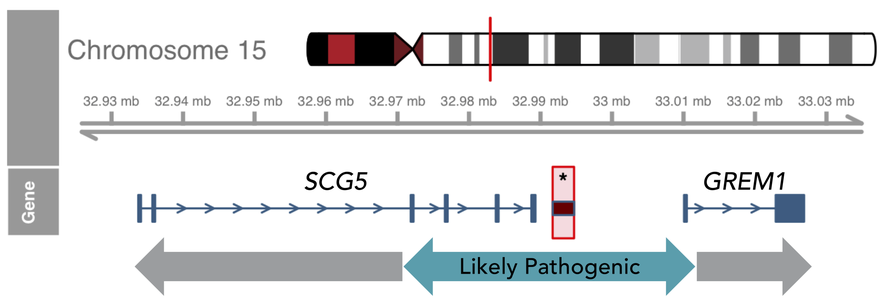
- A duplication of the entire *GREM1* gene has been previously described in an individual who did not have polyposis but was affected with early-onset sigmoid colon carcinoma[4]. That duplication is 57kb and spans the 3' end of the *SCG5* gene (exons 3-6) and the entire *GREM1* gene.
- Probands 5 and 6 have a 60kb duplication with a different 5' breakpoint than the reported 57kb duplication[4].
  - Proband 5 has a personal history of colon polyps and a family history of colorectal cancer and polyps.
  - Proband 6 reported no personal and family history of colorectal cancer or polyps.

### A *GREM1* whole-gene triplication

- Two independent studies have found a significant increase in *GREM1* mRNA expression in HMPS or polyposis-affected individuals over unaffected controls[1,2]. The difference in expression between affected and control may be as little as 2-fold[2].
- We identified a >650kb triplication that encompassed the whole *GREM1* gene, which doubles the gene dosage from 2 copies to 4 copies.
  - Proband 12 (over age 40) has reported colon polyps but no personal or family history of colorectal cancer or polyps.
- The clinical phenotypes of the whole-gene *GREM1* duplication and triplication carriers suggest that a detected gene dosage increase (i.e. *GREM1* copy-number gain) may not translate directly into a proportionate increase in *GREM1* expression and/or the molecular etiology of *GREM1*-associated colorectal cancer and polyps may include other forms of gene dysregulation.
  - This is supported by the spatial mis-expression of *GREM1* reported in HMPS patients and in mice model of *GREM1*-induced tumorigenesis[1,7].

**Figure 2. General classification guideline for *GREM1* copy number gain.**

A duplication ≤40kb that encompasses the putative enhancer region is considered likely pathogenic. A larger duplication that spans the whole *GREM1* gene or beyond the 3′ half of the *SCG5* gene is considered a variant of uncertain significance.




## Conclusions

- Colorectal cancer and polyp phenotypes are seen in *GREM1* carriers with duplications that are limited in size (≤40 kb) and centered around the proposed enhancer. This is consistent with the hypothesis that tandem enhancer duplication may be the pathogenic structural rearrangement[1].
- Carriers of duplications that span the whole *GREM1* gene have less significant health history of colorectal cancer and polyps, as compared to the 24kb and 40kb duplications of the putative enhancer.
- Increase in *GREM1* whole-gene copy number is not an absolute predictor of pathogenicity.