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Cancer Prev Res (Phila). Author manuscript; available in PMC 2015 August 01.

Published in final edited form as:
Cancer Prev Res (Phila). 2014 August ; 7(8): 805–812. doi:10.1158/1940-6207.CAPR-14-0108.

Inactivating Mutation in the Prostaglandin Transporter Gene, *SLCO2A1*, Associated With Familial Digital Clubbing, Colon Neoplasia, and NSAID Resistance

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Abstract

HPGD and *SLCO2A1* genes encode components of the prostaglandin catabolic pathway, *HPGD* encoding the degradative enzyme 15-PGDH, and *SLCO2A1* encoding the prostaglandin transporter PGT that brings substrate to 15-PGDH. *HPGD* null mice show increased PGE_2, marked susceptibility to developing colon tumors, and resistance to colon tumor prevention by NSAIDs. But in humans, *HPGD* and *SLCO2A1* mutations have only been associated with familial

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digital clubbing. We here characterize a family with digital clubbing and early onset colon neoplasia. Whole exome sequencing identified a heterozygous nonsense mutation (G104X) in the *SLCO2A1* gene segregating in three males with digital clubbing. Two of these males further demonstrated notably early onset colon neoplasia, one with an early onset colon cancer and another with an early onset sessile serrated colon adenoma. Two females also carried the mutation, and both these women developed sessile serrated colon adenomas without any digital clubbing. Males with clubbing also showed marked elevations in the levels of urinary prostaglandin E_2 metabolite, PGE-M; whereas, female mutation carriers were in the normal range. Furthermore, in the male proband urinary PGE-M remained markedly elevated during NSAID treatment with either celecoxib or sulindac. Thus, in this human kindred, a null *SLCO2A1* allele mimics the phenotype of the related HPGD null mouse, with increased prostaglandin levels that cannot be normalized by NSAID therapy, plus with increased colon neoplasia. The development of early onset colon neoplasia in male and female human *SLCO2A1* mutation carriers suggests that disordered prostaglandin catabolism can mediate inherited susceptibility to colon neoplasia in man.

INTRODUCTION

Digital clubbing is characterized by focal bulbous enlargement of the terminal segments of fingers and/or toes, and in sporadic form is associated with a variety of clinical conditions including cancer, cardiovascular disease and inflammatory disorders (1). Familial digital clubbing is a rare inherited genetic disorder that shows an autosomal recessive or a dominant inheritance pattern with variable penetrance, and may occur as part of the syndrome of primary hypertrophic osteoarthropathy (PHO) characterized by periostosis, pachydermia, and digital clubbing (1). In the familial form, a significant gender bias has been noted where affected males predominate (7:1 male: female ratio) (2, 3). Inherited genetic defects in components of the prostaglandin degradation pathway have been associated with familial PHO/digital clubbing in diverse patient populations from around the world (1–14). These include homozygous loss-of-function mutations in the *HPGD* gene encoding the prostaglandin degrading enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) (4–8, 13), and both heterozygous and homozygous inactivating mutations in the solute carrier organic anion transporter family, member 2A1 (*SLCO2A1*) gene that encodes the principal prostaglandin transporter (PGT) that mediates prostaglandin uptake into the cell and hence provides substrate for degradation by 15-PGDH (9–12, 14–19).

Numerous studies have demonstrated an important link between elevated levels of prostaglandin E_2 (PGE_2) and the development of colorectal neoplasia (20–23). PGE_2 can stimulate cell proliferation, angiogenesis and motility while inhibiting apoptosis and immune surveillance (24). Reduced levels of both PGT and 15-PGDH commonly occur in colorectal neoplasia and are believed to contribute to elevated levels of PGE_2 in the microenvironment thereby contributing to the pathogenesis of colorectal neoplasia (15, 17, 22, 25–28). Furthermore, there is considerable evidence that inhibitors of PGE_2 production protect against colorectal neoplasia in humans (20, 29–32). This biology has also been demonstrated in murine models, with *HPGD* knockout mice showing increased colon PGE_2 levels concomitant with being rendered strongly susceptible to induction of colon neoplasms

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(26, 27). Moreover, the *HPGD* knockout mouse phenotypes of increased colon tumors and increased colon PGE_2 levels are resistant to correction when this mouse is treated with nonsteroidal anti-inflammatory drugs (NSAIDs), prototypic inhibitors of PGE_2 production (27). In humans though, colon neoplasia has not been previously characterized in kindreds with germline lesions in *HPGD* or in *SLCO2A1*. Here we identify a multi-generational French-Canadian family presenting with male-restricted digital clubbing accompanied by colon neoplasia. Using whole-exome sequencing, we identify an inactivating mutation in the *SLCO2A1* gene as the underlying pathogenetic cause of digital clubbing in this family, and further demonstrate the mutation's association with colon neoplasia and with metabolic resistance to NSAIDs.

MATERIALS AND METHODS

Patient samples, DNA extraction, and medical record and pathology review

The proband and family members were enrolled into the IRB approved CWRU Colon Neoplasia Sibling Study (33). A written consent was obtained from the family members for blood collection to extract, store and analyze genetic material. Also, wherever applicable, a written consent was obtained from family members for collection of medical records including photographs, smoking history, cancer diagnoses, surgery and histopathology records, x-ray and any previous clinical genetic testing.

Review of the proband's (1867-01) medical records confirmed development of adenocarcinoma of the sigmoid colon cancer at age 48. Subsequent follow up colonoscopy examinations did not reveal colonic polyps. Clinical genetic testing of the proband included microsatellite instability analysis of the tumor, and germline sequence analysis of the *MSH2, MLH1, MSH6, PMS2,* and *APC* genes, all of which were normal. Medical and pathology record review of the proband's family members who underwent colonoscopy screening showed multiple members with findings of: no polyps at ages 60 and 79 for proband's father 1867-21; 2 sessile serrated adenomatous polyps at age 54 for proband's sister, 1867-02; 1 sessile serrated adenomatous polyp at age 57 for proband's sister, 1867-03; and a sessile serrated adenomatous polyp at age 24 for proband's nephew, 1867-23.

DNA was extracted from whole blood using the Gentra Puregene Blood kit according to manufacturer's instructions (Qiagen Inc, Valencia, CA) for family members. A second sample of blood was collected and sent to the CLIA approved clinical molecular laboratory University Hospitals Case Medical Center for retesting and verification of the G104X mutation in *SLCO2A1* gene using Sanger sequencing methods as described below.

Whole exome capture and deep sequencing

Target capture, library preparation, and deep sequencing were performed by the Oklahoma Medical Research Foundation Next Generation DNA Sequencing Core Facility (Oklahoma City, OK). Target sequence enrichments were performed using the Illumina TruSeq Exome Enrichment kit as per the manufacturer's protocols (Illumina Inc, San Diego, CA). Briefly, sample DNA's were quantified using a picogreen fluorometric assay and 3μg of genomic DNA were randomly sheared to an average size of 300bp using a Covaris S2 sonicator

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(Covaris Inc, Woburn, MA). Sonicated DNA was then end-repaired, A-tailed, and ligated with indexed paired-end Illumina adapters. Target capture was performed on DNA pooled from 6 indexed samples, following which the captured library was PCR amplified for 10 cycles to enrich for target genomic regions. The captured libraries were precisely quantified using a qPCR-based Kapa Biosystems library quantification kit (Kapa Biosystems Inc, Woburn, MA) on a Roche Lightcycler 480 (Roche Applied Science, Indianapolis, IN). Deep sequencing of the capture enriched pools was performed on an Illumina HiSeq 2000 instrument with 100bp, paired-end reads to an average read-depth of 70× per sample.

Read mapping, variant detection and annotation

Burrows-Wheeler Aligner (BWA) (34) or Short Oligonucletide Analysis Package (SOAP) algorithms (35) were used to align individual 100bp reads from the raw FASTQ files to the human reference genome (build hg19). Following the conversion of aligned reads in to binary Sequence Alignment/Map (BAM) format, coverage metrics of target bases were calculated using the Picard algorithm (http://samtools.sourceforge.net). On average, Picard metrics showed >80% of the target bases covered at 20× read-depth for the samples, with ~6% of target bases showing no coverage. Next, sequence variations (both single nucleotide and insertion/deletion) in the germline of respective samples were detected using three variant calling algorithms including, SOAPsnp (35), Genome Analysis Toolkit (GATK) (36), and mPILEUP (37). Genomic variants were mapped to the human transcriptome reference database (RefSeq, build hg19) using a variant annotation tool developed in house (SLATE), that identifies variants mapping to gene coding regions and splice-sites, including their corresponding positions and codon changes within respective transcripts.

Filtering of variants and identification of gene candidates

Given a dominant inheritance pattern for the disease in this family, we initially identified all variants including, non-synonymous single nucleotide variants (SNV), insertion/deletion variants (indel), and splice-site variants in gene coding regions that were co-segregating in individuals 1867-01, -02, -21, and -23. This resulted in a total of 7292 coding sequence variants. Next, we eliminated variants with a minor allele frequency (MAF) greater than 1% based on the 1000 genome database as well as by clashing against our in-house germline variant database generated from platform-matched whole exome sequencing of germline samples with European ancestry (n>150). The use of platform-matched in-house database additionally aided in eliminating recurrent artifacts or false positives seen in the NGS data. This resulted in a total of 65 rare candidate coding sequence germline variants passing the above filter criteria. Of the 65 variants, 8 were seen in individual 1867-22 who is the mother of the proband, and were subsequently eliminated resulting in 57 variants. Finally, aligned reads mapping to each of the 57 variant genomic loci were manually reviewed using the Integrative Genomics Viewer (Broad Institute) to eliminate obvious sequence artifacts. In the end, 54 variants mapping to the coding regions of 52 candidate genes passed all the filtering criteria. Prostaglandin pathway network analysis was performed on the candidate genes by manual curation as well as by using the Ingenuity Pathway Analysis software package (Ingenuity Systems Inc, Redwood city, CA).

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Sanger sequencing

The primers for amplifying the *SLCO2A1* mutant locus in germline DNA, and primers for amplifying coding regions of *SLCO2A1* from formalin-fixed paraffin-embedded DNA are listed in supplementary Tables S1 and S2. The PCR conditions included 95°C for 4 min, 38 cycles of 95°C for 45s, 62.3°C for 30s and 72°C for 45s. Each reaction was carried out in a 50μl reaction volume using 2.5 U of Fast-TAQ DNA polymerase (Roche Applied Science, Indianapolis, IN) with 25–50ng of template DNA. The PCR products were purified and sequenced using universal M13 forward and reverse primers by Beckman Coulter Genomics, Danvers, MA. Analysis of Sanger sequencing data was performed using Mutation Surveyor software package (SoftGenetics, State College, PA).

Quantification of urinary PGE-M

The major urinary metabolite of PGE_2, 11α-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M), was analyzed using liquid chromatography-mass spectrometry (LC/MS) with slight modifications to the method previously described by Murphey and colleagues (38, 39). Briefly, 1mL urine was converted to the O-methyloxime derivative and purified by C18 solid phase extraction before analysis by LC/MS. LC was performed on a 2.0 × 50 mm, 1.7μm particle Acquity BEH C18 column (Waters Corporation, Milford, MA). Mobile phase A was 95:4.9:0.1 (v/v/v) 5 mM ammonium acetate:acetonitrile:acetic acid, and mobile phase B was 10.0:89.9:0.1 (v/v/v) 5 mM ammonium acetate:acetonitrile:acetic acid. Samples were separated by a gradient of 85–76% of mobile phase A over 6 min at a flow rate of 200μl/min prior to delivery to a ThermoFinnigan TSQ Quantum Vantage triple quadrupole mass spectrometer. Urinary creatinine levels were measured using a test kit from Enzo Life Sciences. The urinary PGE-M level in each sample was normalized using the urinary creatinine level of the sample and expressed in ng/mg creatinine. Levels of urinary PGE-M in normal, healthy men are 10.4 ± 1.5 ng/mg Cr while levels in normal, healthy women are slightly lower averaging 6.0 ± 0.7 ng/mg Cr (38, 39).

RESULTS

Family 1867 demonstrates autosomal male digital clubbing and early onset colon neoplasia

We identified a multi-generational French-Canadian family (family 1867) presenting with digital clubbing restricted to males and showing an autosomal dominant inheritance pattern (Figure 1). Figure 2 shows representative images of the clubbing phenotype in the male proband (1867-01) demonstrating bilateral bulbous enlargement of terminal segments of fingers and toes. The proband's father is also affected by digital clubbing, as by history were the proband's paternal grandfather (Figure 1). Moreover, a nephew of the proband (1867-23) has also developed digital clubbing (Figure 1). Additionally, colon neoplasia was common in the family. In males, colon neoplasia showed early onset, with the proband developing a stage III colon cancer at age 48, and, with nephew of the proband, 1867-23, developing a 0.5cm sessile serrated adenoma at the young age of 24 (Figure 1, Table 1). However, colon neoplasia also developed in female family members. One of the proband's sisters, 1867-02, had two sessile serrated adenomatous polyps at age 54, the largest being 1cm in size. A

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second sister, 1867-03, had a 0.6cm sessile serrated adenoma at age 57. Review of the family's clinical records and molecular genetic testing revealed no evidence of familial adenomatous polyposis (FAP) or hereditary nonpolyposis colorectal cancer (HNPCC).

Identification of inactivating mutation in the SLCO2A1 gene in family 1867

To identify the genetic basis of digital clubbing in this family, we performed whole-exome sequencing of germline DNA available from 6 individuals including, 3 males affected with clubbing (1867-01, -21, -23), 2 females unaffected by clubbing (1867-02,-03), and the mother of the proband (1867-22) (Figure 1). Target capture, deep sequencing, variant calling, and identification of candidate genes were performed as described in the methods section. Analysis of the whole-exome data identified 54 rare germline variants that showed segregation in all 3 males affected with clubbing, and that were additionally all present in the 2 unaffected female siblings (Figure 1). These included 1 frame-shift, 52 missense, and 1 nonsense mutation that mapped to the coding regions of 52 genes. Given prior studies, we first focused on variants mapping to genes involved in prostanoid synthesis or degradation pathways (1–14). The only such mutant gene was *SLCO2A1* that encodes the principal prostaglandin transporter and is involved in the uptake of PGE_2 for degradation (40, 41) in which a nonsense mutation (p.G104X, codon 104) was segregating in family 1867 (Figures 1 and 3). This germline mutation has previously been reported in a single Japanese patient presenting with digital clubbing and periostosis (though with no family history of the disease) (11), and also in a consanguineous Italian family thought to have an autosomal recessive syndrome of digital clubbing, pachydermia, and periostosis (2, 6). These findings strongly suggest that this *SLCO2A1* inactivating mutation is the underlying cause of digital clubbing in male members of family 1867. Notably, in family 1867 all male individuals with clubbing carried one wild-type *SLCO2A1* allele, indicating the mutant allele as being dominant. Moreover, mutant alleles were also carried in two female family members without clubbing as well as in one younger male individual who also lacks clubbing. Thus, the clubbing phenotype appears to be incompletely penetrant, with females in particular resistant to clubbing development.

Increased urinary PGE-M in male individuals with clubbing

Measurements of urinary PGE-M, a stable 15-PGDH pathway end metabolite of PGE_2, are used as an index of systemic PGE_2 levels (25, 38, 39, 42–45). After establishing the mutational status of PGT, we quantified levels of urinary PGE-M in members of family 1867. As shown in Table 1, affected male members with clubbing and the *SLCO2A1* mutation showed greater than 2.5-fold higher levels of urinary PGE-M when compared to levels (10.4 ± 1.5 ng/mg Cr; (38)) reported by our laboratory in normal healthy males. Thus, in males with clubbing, this mutation in the prostaglandin degradation pathway was associated with increased systemic levels of PGE_2. This alteration in PGE metabolism appears to be gender-specific and strongly correlates with the clinical restriction of clubbing to only male mutation carriers. Although smoking can be an exogenous cause of increased urinary PGE-M (42, 44), both the proband (1867-01), and his nephew (1867-23), have never smoked, and these two individuals showed the two highest levels of urinary PGE-M.

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Elevated PGE-M is resistant to NSAID treatment

Cyclooxygenases (COX)-1 and -2 play critical roles in the synthesis of PGE_2 (46). NSAIDs inhibit COX enzymes and thereby suppress PGE_2 biosynthesis. Both dual inhibitors of COX-1 and COX-2 and selective COX-2 inhibitors have been reported to suppress urinary PGE-M levels in humans (38, 39). Accordingly, we tested the potential of COX inhibitors to reverse increased urinary PGE-M levels in the proband (1867-01). We first tested sulindac, a prodrug that is metabolized to sulindac sulfide, a dual inhibitor of COX-1 and COX-2. Treatment with oral sulindac at 150 mg twice daily for 4 weeks was associated with about a 40% decline in urinary PGE-M levels, from 56.40 to 33.72 ng/mg Cr, a level that still remained well above the normal range. We then tested celecoxib, a selective inhibitor of COX-2. Treatment with celecoxib 200 mg twice daily for 6 weeks was associated with a 56% decline in urinary PGE-M levels, from 56.40 to 24.79 ng/mg Cr, which again was still elevated well above normal. Thus, while treatment with these two NSAIDs, that both have known colon tumor preventive properties (31, 47), lowered levels of urinary PGE-M, neither drug could effectively normalize the increase in urinary PGE-M caused by the *SLCO2A1* mutation.

Colon neoplasia is coincident with SLCO2A1 mutation in family 1867

As mentioned above, four members of family 1867 had evidence of colon neoplasia. First, the male proband (1867-01) was diagnosed with stage III colon cancer at the age of 48. Second, the proband's nephew (1867-23) who carries the *SLCO2A1* mutation and has clubbing developed a 0.5 cm sessile serrated adenoma at 24 years of age. Additionally, the proband has two female siblings (1867-02, 1867-03) who both carry the *SLCO2A1* mutation and who also both developed sessile serrated adenomas, although neither demonstrated clubbing (Table 1). The father of the proband (1867-21) underwent colonoscopy at ages 60 and 79, with no evidence of colon neoplasia. The remaining male mutation carrier, 1867-24, age 26, who does not have clubbing has not been evaluated for colon neoplasia. Overall, four first-degree relatives carrying a *SLCO2A1* mutation were affected with colon neoplasia, one with early onset colon cancer, and three with sessile serrated adenomas. We found no evidence for bi-allelic inactivation of the *SLCO2A1* gene in the tumor tissue available from the proband (data not shown). Though our findings suggest that a *SLCO2A1* gene mutation leads to increased systemic levels of PGE_2 and associated clubbing only in males, the pattern of colon neoplasia nonetheless suggests that a mutation in *SLCO2A1* may increase the risk of colon neoplasia in both males and females.

DISCUSSION

Here we identify an autosomal dominant inactivating mutation in the *SLCO2A1* gene (p.G104X) as an underlying cause for male-restricted familial digital clubbing in a French-Canadian family. The finding of male-restricted clubbing in family 1867 is in agreement with prior studies that noted a male predominance of affected individuals with familial PHO/digital clubbing (2, 4, 6, 10, 12). While the underlying mechanisms for gender-restricted penetrance are uncertain, it is known that baseline levels of urinary PGE-M, which reflect systemic PGE_2 levels, are higher in healthy males than females (38). Our findings that a PGT mutation leads to higher levels of urinary PGE-M (and presumptively systemic PGE_2)

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in male mutation carriers than in female mutation carriers suggests one mechanism that could account for gender-specific differences in the incidence of clubbing. Moreover, the dominant phenotype demonstrated by the G104X nonsense mutation suggests that haploinsufficiency of the prostaglandin transporter is sufficient to reduce prostaglandin uptake enough to increase systemic levels of PGE_2 at least in males. Alternatively, the N-terminal fragment encoded by this mutation may have dominant negative function.

The fact that neither treatment with sulindac nor celecoxib led to a normalization of urinary PGE-M levels in the proband is also of interest. As shown in Table 2, while NSAID treatment did lower the proband's levels of urinary PGE-M as compared to baseline, the PGE-M levels still remained significantly above the upper limits seen in normal males. Notably, PGE_2 can induce COX-2 and mPGES-1 in some cell types (48) – a positive feedback loop. We posit that the PGT mutation, by decreasing PGE_2 uptake into the 15-PGDH catabolic pathway, leads to elevated extracellular levels of PGE_2 that results, in turn, in up regulation of enzymes involved in PGE_2 synthesis. Higher doses of NSAIDs may be required to inhibit PGE_2 production in this context. These findings are also consistent with our previous observations in *HPGD* knockout mice, in which treatment with celecoxib also failed to normalize levels of colonic PGE_2 (26, 27). The finding of limited efficacy of NSAIDs in humans and animals with genetic defects in the PGT – 15-PGDH pathway provides strong evidence for the critical role of prostaglandin degradation in regulating PGE_2 levels *in vivo*.

Perhaps the most striking feature of family 1867 is the finding of colon neoplasia in multiple members of the family who carry the *SLCO2A1* mutation, including advanced colon cancer at age 48 in the proband (1867-01), sessile serrated adenomas in two of his sisters, and a sessile serrated adenoma in 1867-23 (nephew of the proband) at the young age of 24 years (Table 1). Sessile serrated adenomas appear to form via a unique molecular pathway (49). Our findings, if confirmed in additional studies, suggest that prostaglandins may play a role in the pathogenesis of sessile serrated adenomas. In support of this possibility, use of aspirin, a known inhibitor of prostaglandin production, has been associated with a reduced risk of serrated polyps (50). It is intriguing that two female carriers of the *SLCO2A1* mutation had a history of sessile serrated adenomas in the absence of elevated levels of urinary PGE-M. Perhaps in females defects in the PGT – 15-PGDH pathway increase PGE_2 locally in the colon mucosa, but less so systemically, thereby increasing the local risk of colon neoplasia in the absence of altered systemic levels of PGE_2. We of course cannot exclude the possibility that the development of sessile serrated adenomas in females without clubbing is a phenocopy that is unrelated to any disorder in prostaglandin metabolism. In prior studies, we have shown that *HPGD* knockout mice, that are defective in prostaglandin degradation, demonstrate both elevated colonic PGE_2 and a marked increase in susceptibility to colon neoplasia (26, 27). Additionally, we have demonstrated the continued development of colonic adenomas during celecoxib treatment in *HPGD* knockout mice and in humans who have below average levels of 15-PGDH in colonic mucosa (27). We now demonstrate a striking concordance in the phenotypes that result from *HPGD* knockout in the mouse and from *SLCO2A1* mutation in a human kindred, which in both instances are associated with increased colon neoplasia and with resistance to NSAIDs. As PGT can transport molecules

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in addition to PGE_2, it remains possible that the *SLCO2A1* mutation increases the risk of colon neoplasia via a prostaglandin-independent mechanism. Regardless of the underlying mechanism(s) that increase the risk of colorectal neoplasia, our observations support offering colon neoplasia screening at a young age to all individuals who carry inactivating mutations in either *HPGD* or *SLCO2A1*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the members of family 1867 participating in this study. This study was supported by NIH grants: RO1 CA130901 (SDM), RO1 CA127306 (SDM), and Case GI SPORE P50 CA150964 (SDM), KO8 CA148980 (K.G.), Career Development Program of Case GI SPORE (P50 CA150964) award to K.G and the New York Crohn's Foundation (AJD).

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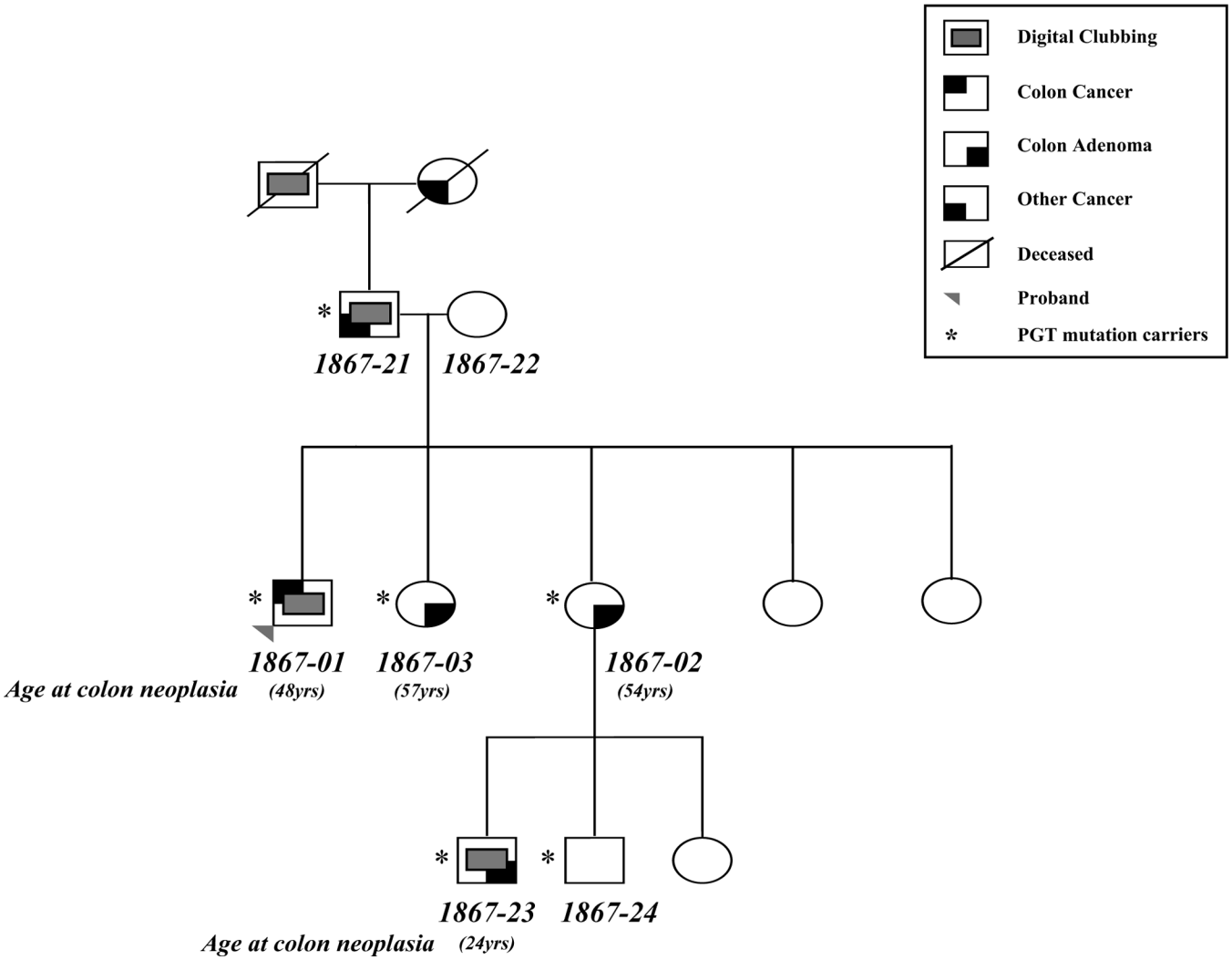

Figure 1.

1867 family pedigree. Open squares represent males, and circles represent females. Whole exome sequencing was performed on individuals 1867-21,-22,-01,-02,-03, and -23. Asterisk (*) indicates individuals carrying the *SLCO2A1* G104X heterozygous non-sense mutation. G104X mutation in individual 1867-24 was identified through direct Sanger sequencing.

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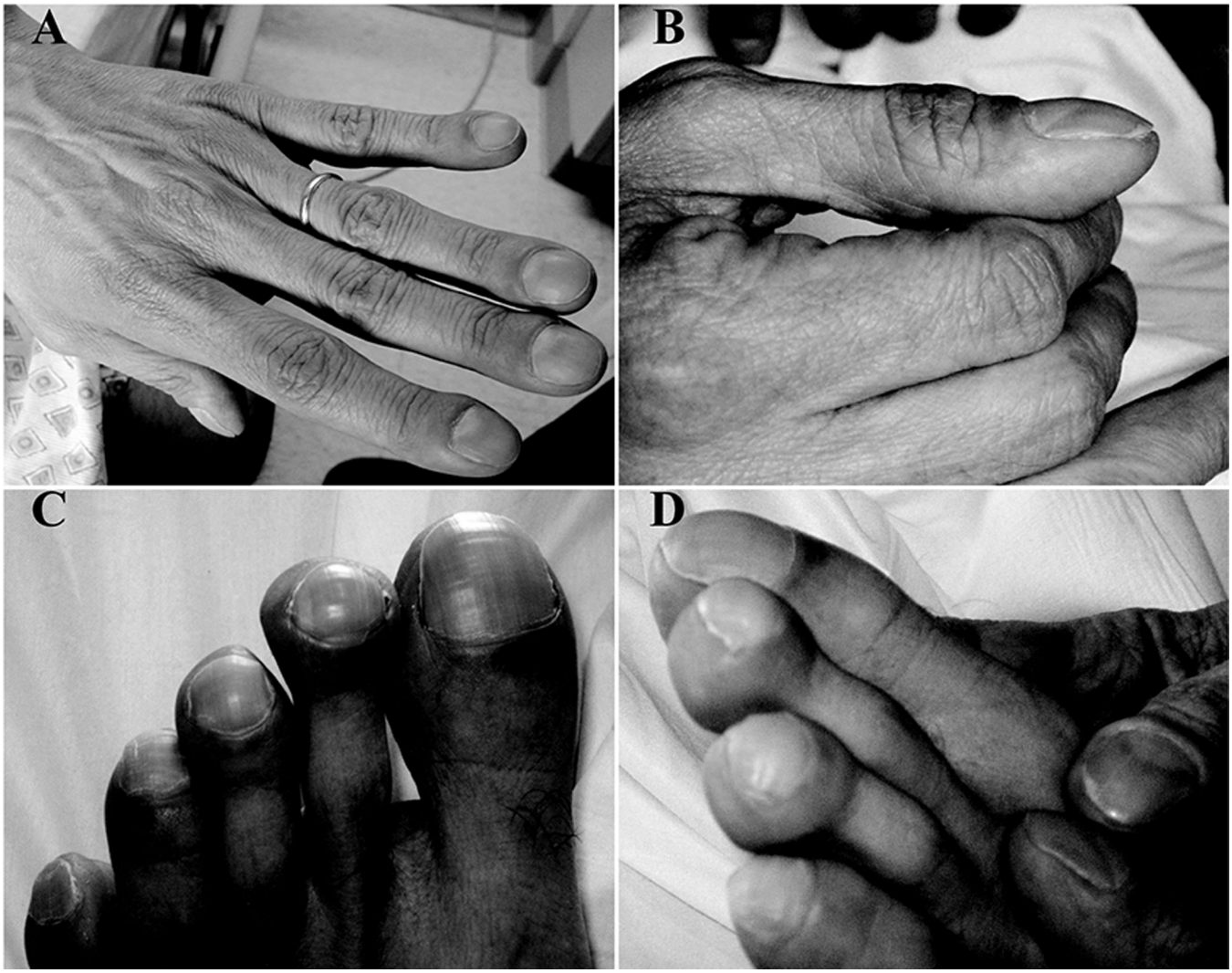

Figure 2.

Clinical manifestation of digital clubbing. A–D, Shown are representative images from individual 1867-01 (proband) demonstrating bulbous enlargement of fingers (A, B) and toes (C, D).

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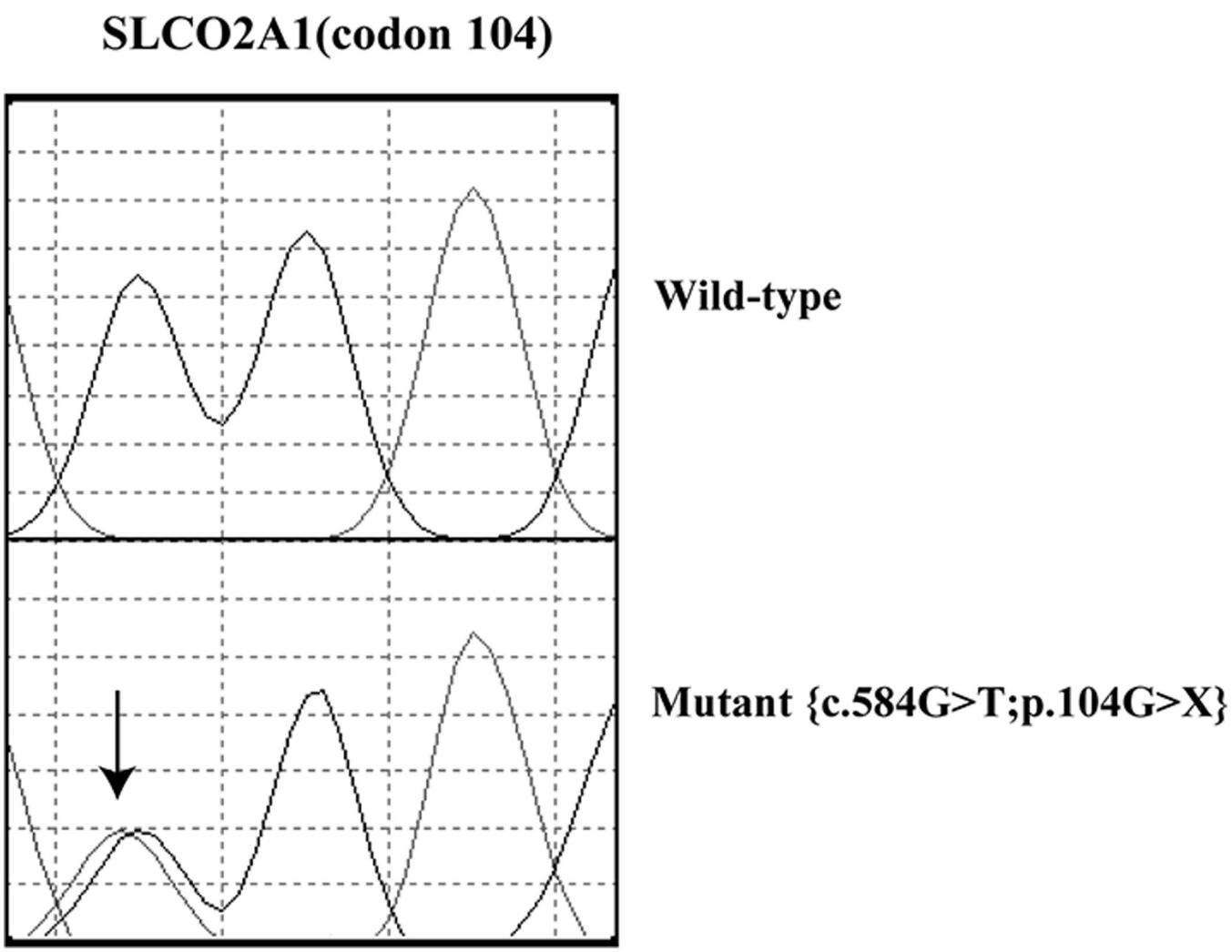

Figure 3.

Representative chromatograms from direct Sanger sequencing of *SLCO2A1* codon 104 position in germline DNA showing wild-type (GGA) sequence, and heterozygous mutant (TGA) position (arrow).

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Table 1

Baseline concentrations of urinary Prostaglandin E metabolite (PGE-M) in family members.

Individual	Age at follow-up (yrs)	Gender	Relationship	Digital clubbing	Smoking status (pack-years)	Colon neoplasia (age at D_x)	PGE-M (ng/mg Cr)
1867-01	52	Male	Proband	Yes	Never smoker	Sigmoid Colon cancer (48)	56.40
1867-21	88	Male	Father of proband	Yes	Former smoker[a] (93)	None	26.94
1867-23	24	Male	Son of 1867-02	Yes	Never smoker	Sessile serrated adenoma, 0.5cm (24)	33.96
1867-24	26	Male	Son of 1867-02	No	Former smoker (4)	Unknown	Unknown
1867-02	56	Female	Sister of proband	No	Never smoker	Two sessile serrated adenomas,1cm and 0.4cm (54)	3.09
1867-03	60	Female	Sister of proband	No	Never smoker	Sessile serrated adenoma,0.6cm (57)	5.94
1867-22	87	Female	Mother of proband	No	Never smoker	Unknown	13.31

[a]Smoked from age 20–51 yrs.

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Table 2

Urinary concentrations of PGE-M in proband 1867-01 following sequential treatment with Sulindac and Celecoxib, respectively.

Treatment	Duration of treatment	PGE-M (ng/mg Cr)
None (baseline from Table1)	Not applicable	56.40
Sulindac (150mg, twice daily)	4 weeks	33.72
Celecoxib (200mg, twice daily)	6 weeks	24.79