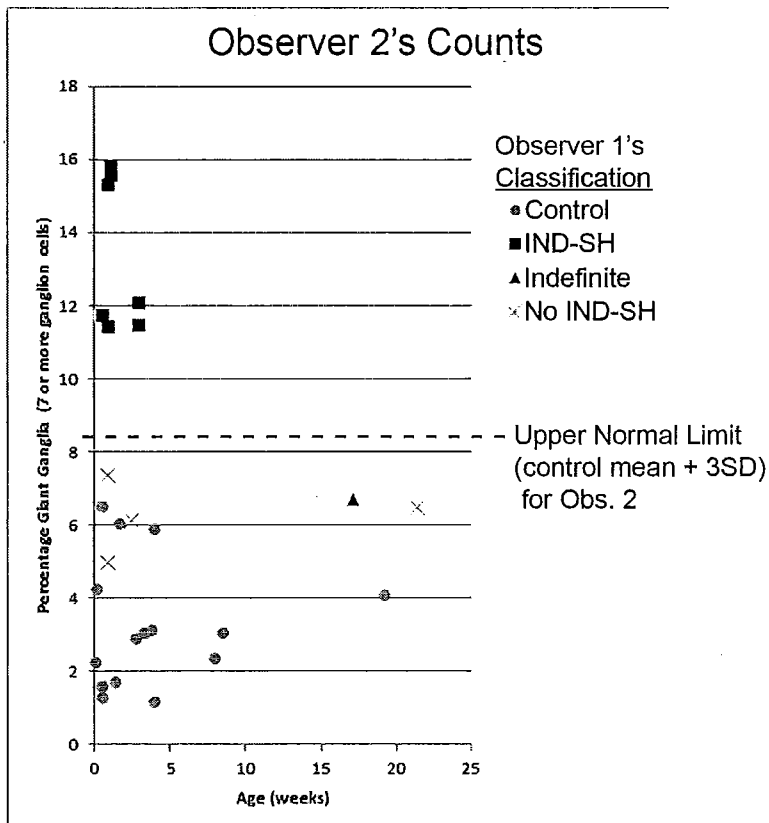


Figure S1



Supplemental Figure 1: Relative abundance of giant submucosal ganglia versus age as determined by Observer 2 for the controls and a subset of HSCR patients independently analyzed by Observer 1. The symbols indicate the groups defined by Observer 1 (see Figure 4). The dashed line indicates the upper normal limit (8.3%) for Observer 2, based on his control mean + 3SD. The same 7 patients were classified independently as IND-SH by both observers.

Supplemental Table 1: Criteria used to count submucosal ganglion cells

1. All areas counted should be situated within the submucosa
2. An area of dark brown perikaryal staining in a cell that contains a nucleus (a nucleus must be counterstained blue with or without superimposed brown immunoreactivity and have sharp contours; a lucent area or semicircular outline is not sufficient) <ul style="list-style-type: none">- Granular stain must cover the nucleus OR encircle at least 50% of circumference of the nucleus- AND at least some cytoplasmic granular brown staining must be present
3. Overlapping or continuous areas of dark brown staining, in the presence of 2 distinct nuclei and cell membranes, should be counted as 2 cell bodies
4. If there is any ambiguity about the presence of a nucleus, the cell should not be included

Supplemental Table 2: Intra-observer variability

	Full-circumference total submucosal ganglion cell count*				Relative Percent Difference (100*difference/mean)	
	A	B	C	D	Intra-obs 1	Intra-obs 2
Slide	Observer 1 Count 1	Observer 1 Count 2	Observer 2, Count 1	Observer 2 Count 2	(A v B)	(C v D)
A	434	451	514	438	3.84	15.97
B	435	436	415	413	0.23	0.48
C	471	402	519	488	15.81	6.16
D	326	271	429	376	18.43	13.17
E	480	436	559	539	9.61	3.64
				Mean +/- SE	9.58 ± 3.45	7.88 ± 2.91

*For each observer, counts 1 and 2 were separated by more than 300 days. Abbreviations: obs, observer; SE, standard error of the mean.

Supplemental Table 3: Inter-observer variability

Slide	Full-circumference total submucosal ganglion cell count*			Difference (B - A)	RPD A v B
	Observer 1 A	Observer 2 B	Mean		
Control 1	261	311	286	50	16.08
2	488	542	515	54	9.96
3	246	335	290.5	89	26.57
4	337	408	372.5	71	17.40
5	435	413	424	-22	5.33
6	419	512	465.5	93	18.16
7	434	438	436	4	0.91
8	326	376	351	50	13.30
9	289	368	328.5	79	21.47
13	207	432	319.5	225	52.08
16	423	768	595.5	345	44.92
18	132	474	303	342	72.15
22	56	199	127.5	143	71.86
23	205	419	312	214	51.07
24	148	287	217.5	139	48.43
HSCR 1	764	868	816	104	11.98
HSCR 4	796	1053	924.5	257	24.41
HSCR 10	329	545	437	216	39.63
HSCR 12	608	584	596	-24	-4.11
HSCR 18	403	462	432.5	59	12.77
HSCR 23	480	539	509.5	59	10.95
HSCR 28	489	616	552.5	127	20.62
HSCR 31	599	939	769	340	36.21
HSCR 33	1105	1275	1190	170	13.33
HSCR 35	612	1106	859	494	44.67
HSCR 36	471	488	479.5	17	3.48
HSCR 37	512	574	543	62	10.80
HSCR 38	312	634	473	322	50.79
HSCR 42	578	801	689.5	223	27.84
Mean					26.66
SE					3.81

Abbreviations: RPD, relative percentage difference (100 * difference/mean); SE, standard error of the mean