Volume 48(1-4):7-12, 2004
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Differences in distribution of the 210 kDa neurofilament subunit and the S-100 protein in the small intestine of a human fetus with trisomy 21 and in that of a normal fetus of the same age

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ABSTRACT The enteric nervous system of the small intestine of a 22-week-old male fetus with trisomy 21 was examined and compared with gut specimens from a fetus with normal karyotype at the same developmental stage. After the therapeutic termination of the pregnancies, paraffin sections and whole-mounts were prepared, which were processed for histology and immunohistochemistry, with the use of antibodies against the 210 kDA neurofilament subunit and the glial marker S-100 protein. The reduced length of the small intestine, the histologically observed fewer and shorter villi and the frequently appearing pseudostratified epithelium indicate an overall delay in the intestinal development in the trisomic fetus. Both the S-100 protein-immunopositive glial cells and the neurofilament protein-immunopositive nerve cells were distributed differentially in the gut specimens of the trisomic fetus and in the fetus with normal karyotype. While the immunohistochemical expression of the S-100 protein differed only in the circular axis of the gut wall, the distribution of the neurofilament protein-immunoreactive nerve cells also differed along the longitudinal axis of the gastrointestinal tract. Not only the distribution, but also the morphology of the neurofilament protein-immunoreactive myenteric ganglion cells differed in the trisomic fetus as compared with the normal one. The neurofilament protein-immunopositive ganglion cells of the normal fetus possessed lamellar dendrites and one long axon, while the ganglion cells of the trisomic fetus did not exhibit special morphological characteristics. These observations suggest that the enteric nervous system of the fetus with trisomy 21 is involved in the overall delay of the gut development.
Acta Biol Szeged 48(1-4):7-12 (2004)

KEY WORDS

fetal intestine
trisomy 21
neurofilament
S-100 protein

Trisomy 21 is the most common autosomal aneuploidy compatible with postnatal survival. It occurs in an average of 1 in 700 live births (Hassold et al. 1996). The presence of trisomy 21 is regarded as a significant risk factor for Hirschsprung disease (HSCR) and for a spectrum of defects in gastrointestinal motility (Epstein et al. 1991; Holschneider et al. 1994; Cohen 1999). In consequence of the wide and variable set of clinical features in a given individual, trisomy 21 is viewed as a predisposing factor, rather than as a causative factor for these diseases (Quinn et al. 1994). The gene catalog of chromosome 21 contains at least 10 kinases, 5 cell adhesion molecules and a number of transcription factors (Hattori et al. 2000). It is possible that an extra copy of these genes results in an overall delay in intestinal development as a primary disorder, and the delayed maturation of the gastrointestinal tract may then predispose or contribute to the development of disorders such as HSCR, intestinal obstruction or enterocolitis in infants with trisomy 21.

Murine trisomy 16 is accompanied by a complex genetic insult similar to that in human beings with segmental trisomy 21 (Pletcher et al. 2001). Investigations of mice with trisomy 16 have demonstrated a delayed development in the enteric nervous system (ENS) of the stomach and in the intestine (Li et al. 2000). We report here on a comparative study of a 22-week-old male fetus with prenatally diagnosed trisomy 21 and a normal fetus at the same developmental stage. Histological features typical of a younger fetal age were revealed in the fetus with trisomy 21, suggesting an overall delay in the development of the intestine. To establish whether or not the ENS was involved in the delayed development, we applied immunohistochemistry, using primary antibodies against a neurofilament (NF) subunit protein and against S-100 protein. Both antigens have been widely used to study the architecture of the ENS both in normal (Eaker 1997; Fekete et al. 1999) and in pathological (Yanagihara et al.

Accepted July 21, 2004
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1992; Deguchi et al. 1993) cases. It is well documented that cytoskeletal proteins such as NF proteins are developmentally regulated (Faussone-Pellegrini et al. 1999) and are suitable for the morphological characterization of neuronal subgroups (Román et al. 2001; Brehmer et al. 2002). These literature data indicate that NF proteins are potential markers for the study of neuronal disorders or developmental disturbances affecting morphologically distinct neuronal subgroups.

The glial marker S-100 protein furnishes information on the distribution of glial cells, which create the local microenvironment necessary for neuronal development (Bush et al. 1998; Giaroni et al. 1998; Fekete et al. 1999). The differences in the distribution and morphological appearance of the NF and S-100 protein-immunoreactive cells observed in this comparative study indicate an intestinal segment-dependent impairment in the development of the myenteric ganglion cells and the enteric glia in the ENS of the fetus with trisomy 21. To the best of our knowledge, this is the first reported case on a fetus with trisomy 21 with a developmental disorder of the ENS.

Materials and Methods

Human samples and tissue handling

The human study was approved in advance by the University Medical Ethical Committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. For tissue preparation, the small intestines of the fetuses with trisomy 21 or with normal karyotype were obtained immediately following therapeutic abortion performed by the laminaria plus Nalador technique after the intrauterine death of the fetuses. The crown-rump lengths of the fetuses involved in this study were 209 and 212 mm respectively, corresponding to gestational week 22.

The lengths of the unfixed small intestine along the unstretched antimesenteric border from the pylorus to the ileocecal valve were 81 and 95 cm respectively. Selected segments of the small intestine were ligated and distended, using a modified Zamboni fixative (Scheuermann et al. 1987) and fixed overnight at 4°C. The proximal, middle and distal segments were selected at 1 and 40 cm distal to the pylorus and 3 cm oral to the ileocecal junction, respectively.

Small pieces were processed for paraffin sectioning, while larger pieces were used for whole-mount preparations. Paraffin sections were stained with mucicarmine for histological observation, while unstained paraffin sections were incubated together with whole-mounts for immunostaining, using the avidin-biotin technique (Fekete et al. 1999). Anti-S-100 protein (Dakopatts Z 311, final dilution 1:400) and anti-NF 210 kDA subunit (SIGMA clone 52 mo, final dilution 1:200) were used as primary antibodies. Specimens were mounted in PBS-buffered glycerol and viewed in a Leica DMLB light microscope equipped with a Polaroid digital camera.

Results

The small intestine of the 22-week-old human fetus with karyotype 47, XY+21 (Fig. 1) was 15% shorter than the small intestine of the fetus with normal karyotype at the same developmental stage. Histologically, fewer and shorter villi were observed and pseudostratified epithelium was frequently seen on the villous and intervillous epithelium (Fig. 2).

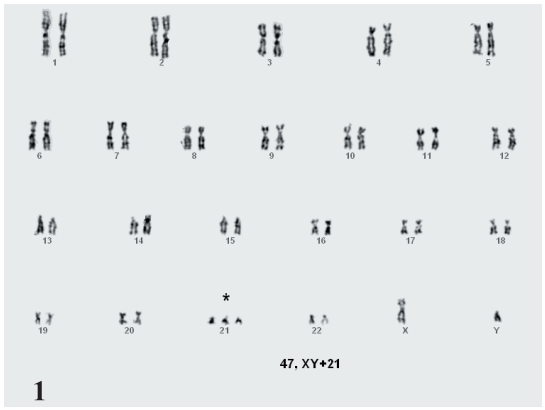
The S-100 protein immunostaining revealed dense glial staining both in the myenteric plexus (MP) and in the submucous plexus (SP; Fig. 3A,B) throughout the whole length of the intestine. However, the S-100 protein staining was always restricted to the outer SP in the fetus with trisomy 21 (Fig. 3B). The number and distribution of the S-100 protein-immunoreactive cells differed greatly in the MP of the small intestines in the two different fetuses under investigation (Fig. 4A,B). The MP in the fetus with normal karyotype was well developed, with large, distinct ganglia (Fig. 4A). The numerous S-100 protein-immunoreactive cells were mostly located in the center of the ganglia (Fig. 4A). In the fetus with trisomy 21, the myenteric ganglia were not distinct (Fig. 4B), and the few, but intensely stained glial cells were spread throughout the MP (Fig. 4B).

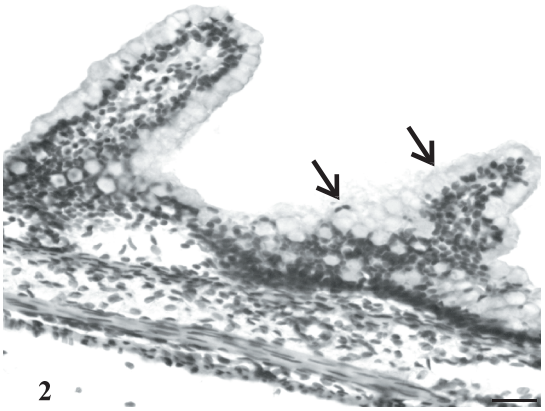
The specimens from the normal fetuses exhibited intense NF immunoreactivity in both the MP and the SP throughout the whole length of the small intestine (Fig. 5A), whereas in those from the fetus with trisomy 21, the immunostaining was restricted to the MP (Fig. 5B). When the distributions of the NF-immunoreactive myenteric ganglion cells were compared along the longitudinal axis of the small intestine, a uniform distribution was revealed in the normal, and a segment-specific different distribution in the trisomic fetal intestine (Fig. 6A-D). The most proximal segments of both intestines were characterized by intense NF immunoreactivity (Fig. 6A,B). However, a semiquantitative assessment demonstrated approximately 50% more NF-immunoreactive ganglion cells in this intestinal segment of the normal fetus. The ganglion cells were not only more numerous here, but also displayed a characteristic neuronal morphology (Fig. 6A), with several lamellar dendrites and one axon.

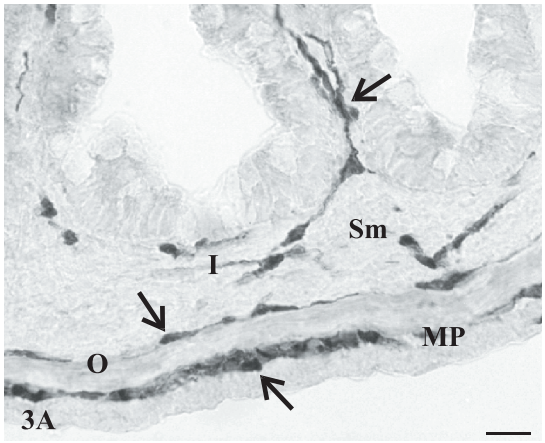
In contrast the NF-immunoreactive ganglion cells in the fetus with trisomy 21 did not exhibit any specialized morphological character: they had only single axons differentiated. Unstained ganglion cells were seen throughout the whole length of the small intestine (Fig. 6C) both in the normal and the trisomic fetus.

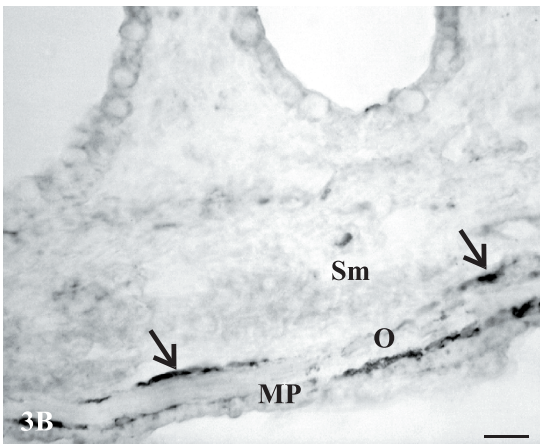
Discussion

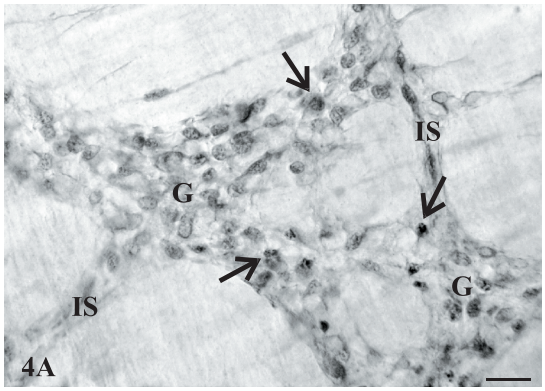
The length of the small intestine of the fetus with trisomy 21 was 81 cm, which corresponds to an 18-week-old (Weaver et al. 1991) rather than a 22-week-old fetal intestine, for which a length of 95 cm was measured. The histological features in the fetus with trisomy 21 were also characteristic of a younger











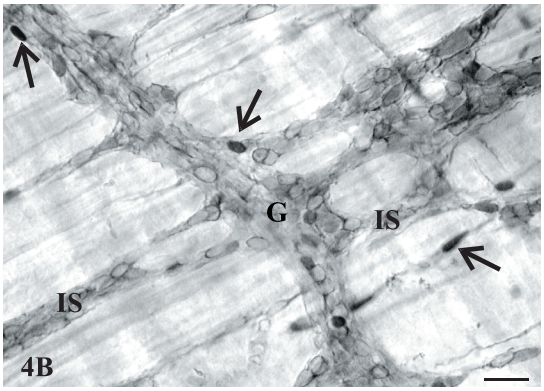


Figure 1. Karyotype of the 22-week-old fetus with trisomy 21 investigated in the present report. Trisomy 21 is indicated by an asterisk.
Figure 2. Mucicarmine-stained paraffin section of the small intestine of the 22-week-old fetus with trisomy 21. Arrows indicate stratified epithelium. Scale bar: 50 μm
Figure 3. Cross-sections of the paraffin-embedded small intestines of the 22-week-old human fetuses processed for S-100 protein immunocytochemistry. Stained glial cells (arrows) are seen in both the myenteric plexus (MP) and in the inner (I) and outer (O) submucous plexus of the normal human fetus (A). In the trisomic fetus (B), immunoreactive cells (arrows) appeared in the myenteric plexus (MP) and in the outer submucous plexus (O). Sm: the submucous layer of the intestinal wall. Scale bars: 40 μm
Figure 4. Whole-mount preparations of the myenteric plexuses from the small intestines of the 22-week-old human fetuses processed for S-100 protein immunocytochemistry. Most of the S-100 protein-immunoreactive glial cells (arrows) accumulate in the center of the large myenteric ganglia (G). Stained cells are not seen in the internodal segments (IS) of the myenteric plexus in the normal fetus (A). Stained glial cells (arrows) in the myenteric plexus of the trisomic fetus (B) are located on the periphery of the ganglia (G), which are not clearly distinct from the internodal segments (IS). Scale bars: 15 μm

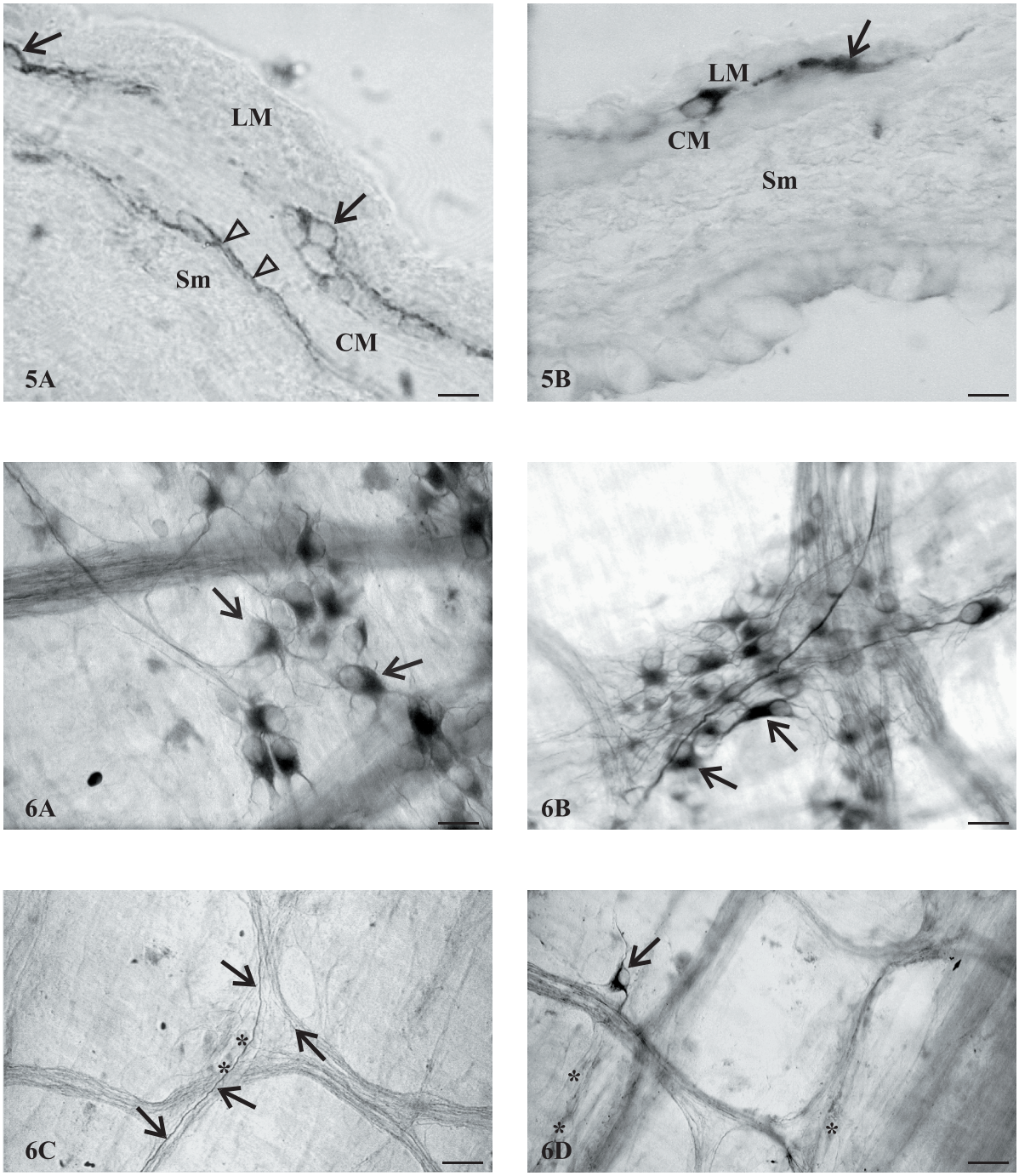


Figure 5. Cross-sections of the paraffin-embedded small intestines of the 22-week-old human fetuses stained with an antibody against the 210 kDA neurofilament subunit protein. Stained ganglion cells and fibers are seen in both the myenteric plexus (arrows) and in the submucous plexus (arrowheads) of the normal human fetus (A). In the trisomic fetus (B), intensely stained ganglion cells and fibers (arrows) were restricted to the myenteric plexus. LM: longitudinal muscle, CM: circular muscle, Sm: submucous layer of the intestinal wall Scale bars: 20 μm

Figure 6. Whole-mount preparations of the myenteric plexuses from the small intestines of the 22-week-old human fetuses stained with an antibody against the 210 kDA neurofilament subunit protein. Numerous stained ganglion cells in the most proximal segment of the small intestine of the normal fetus (A) exhibit a characteristic neuronal morphology, with lamellar dendrites and one axon. Several stained ganglion cells (arrows) are seen in the most proximal segments of the small intestine of the trisomic fetus (B); these ganglion cells did not indicate a definite morphological type. No immunoreactive neurons are seen in the middle segment of the small intestine of the trisomic fetus (C). Asterisks indicate unstained ganglion cells. Several stained cells (arrow) and fibers are seen in the most distal segment of the small intestine of the trisomic fetus. Asterisks indicate unstained ganglion cells. Scale bars represent 10 μm in A and B, 15 μm in C and D.

fetal age (Klein 1989), indicating a severe growth impairment and an overall delay in the development of the gastrointestinal system. To determine whether or not the ENS was involved in the developmental impairment revealed in the small intestine of the fetus with trisomy 21, we looked for cytoskeletal markers that indicate the degree of differentiation attained by the glial and neuronal cells by week 22 of gestation. Cytoskeletal proteins gradually accumulate in the cells in parallel with the organization of the cytoskeletal structures which mediate the important neural events, including the growth of the nerve processes and the initiation of axonal transport (Lasek et al. 1983). Immature cells in the mouse (Faussone-Pellegrini 1999) and in the human (Eaker et al. 1993) intestine express α-internexin and peripherin, while the expression of different NF subunits is related to neuronal differentiation (Bishop et al. 1985; Eaker et al. 1990). Accordingly, α–internexin may be considered a good marker for neuronal immaturity, while NF subunit proteins may be good markers for neuronal maturity. We chose the NF 210 kDA subunit protein in the present comparative study. Although, NF immunostaining demonstrates only a minority of the total enteric neuronal population (Brehmer et al. 2002), the suitability of NF immunohistochemistry for the study of morphologically defined neuron types (Román et al. 2001; Brehmer et al. 2002) makes it an appropriate tool with which to distinguish between neurons via the level of morphological maturity and hence to confirm delayed neuronal differentiation.

Our previous results (Fekete et al. 1995) led us to utilize S-100 protein immunoreactivity as a glial marker in the small intestine of the trisomic fetus and in the fetus with normal karyotype. Differences in immunohistochemical expression were revealed in both the glial marker S-100 protein and the neuronal marker NF 210kDA subunit. As concerns the circular axis of the intestine, the S-100 protein-immunoreactive glial cells were restricted to the outer SP located near the circular layer of the external muscle (Timmermans et al. 2001), whereas in the intestine of the normal fetus the S-100 protein-immunoreactive cells were widely distributed throughout the whole thickness of the submucous layer.

The NF-immunoreactive nerve cells displayed a different distribution not only in the circular axis, but also along the longitudinal axis of the small intestine. The segment-specific differences in NF subunit staining may be consequences of the close temporal and spatial interrelationship between the gut growth and neural maturation. It is well established (Bagyánszki et al. 2002) that the histodifferentiation of the human gut proceeds in an oro-anal wave that is met by an anal-oro wave, which results in a developmentally advanced environment in the foregut and in the hindgut relative to that encountered in the midgut. In the present case, in which the presence of 21 trisomy resulted in an overall growth impairment, the level of development of the middle segment was much behind that of the normal intestine, none of the ganglion cells in this part of the intestine exhibiting NF immunoreactivity. The immunohistochemical expression of NF did not reveal a difference only in the distribution of the NF-immunoreactive neurons: the stained cells in the trisomic and in the normal fetal intestine also differed in their morphology. Numerous immunoreactive ganglion cells in the most proximal segment of the small intestine of the normal fetus demonstrated the morphology of the typical type I neurons in the Stach classification (Stach 1989); in contrast, the ganglion cells in the trisomic fetus did not reach this level of morphological differentiation, and also failed to express immunoreactivity against neuronal markers such as NF protein.

These observations indicate that not only the intestinal wall, but also the ENS is involved in the overall delay of the development of the gastrointestinal tract in the fetus with trisomy 21. The delayed development of the ENS may lead to symptoms of HSCR, intestinal obstruction or enterocolitis in infants with trisomy 21.

References

Bagyánszki M, Kovács ÉG, Resch BÁ, Román V, Resch BE, Fekete É (2002) Computer-aided morphometric analysis of the developing concentric structure of the human fetal intestinal tube. Histol Histopathol 17:731-737.

Bichop AE, Carlei F, Lee V, Trojanovski J, Marangos PJ, Dahl D, Polak JM (1985) Combined immunostainig of neurofilaments, neuron specific enolase, GFAP and S-100. A possible mean for assessing the morphological and functional status of the enteric nervous system. Histochemistry 8: 293-297.

Brehmer A, Schrödl F, Neuhuber W (2002) Morphological phenotyping of enteric neurons using neurofilament immunohistochemistry renders chemical phenotyping more precise in porcine ileum. Histochem Cell Biol 117:257-263.

Bush TG, Savidge TC, Freeman TC, Cox HJ, Campbell EA, Mucke L, Johnson MH, Sofroniew MV (1998) Fulminant jejuno-ileitis following ablation of enteric glia in adult transgenic mice. Cell 93:189-201.

Deguchi E, Iwai N, Goto Y, Yanagihara J, Fushiki S (1993) An immunohistochemical study of neurofilament and microtubule-associated Tau protein in the enteric innervation in Hirschprung's disease. J Ped Surg 28:886-890.

Cohen WI (1999) Health care guidelines for individuals with Down syndrome: 1999 revision. Down Syndrome Q. 4:1-16.

Eaker EY, Shaw G, Sninsky CA (1990) Neurofilament immunoreactivity in myenteric neurons differs from that found in the central nervous system. Gastroenterology 99:1364-1371.

Eaker EZ, Sallustio JE, Harris JM, Shaw G (1993) Myenteric plexus neurons have developmentally acquired differences int he medium molecular weight subunit of neurofilament protein. Neuroscience 53:561-570.

Eaker EY (1997) Neurofilament and intermediate filament immunoreactivity in human intestinal myenteric neurons. Dig Dis Sci 42:1926-1932.

Epstein CJ (1991) Protocols to establish genotype-phenotype correlations in Down syndrome. Am J Hum Genet 49:207-235.

Faussone-Pellegrini M-S, Matini P, DeFelici M (1999) The cytoskeleton of the myenteric neurons during murine embryonic life. Anat Embriol 199:459-469.

Fekete É, Resch BÁ, Benedeczky I (1995) Histochemical and ultrastructural features of the developing enteric nervous system of the human fetal small intestine. Histol Histopathol 10:127-134.

Fekete É, Timmermans JP, Bogers JJ, Resch BA, Van Marck E, Scheuermann DW (1999) Different distribution of S-100 protein and glial fibrillary acidic protein (GFAP) immunoreactive cells and their relations with

nitrergic neurons in the human fetal small intestine. Histol Histopathol 14:785-790.

Giaroni C, De Ponti F, Cosentino M, Lecchini S, Frigo G (1999) Plasticity in the enteric nervous system. Gastroenterology 117:1438-58.

Hassold T, Merrill M, Adkins K, Freeman S (1995) Recombination and maternal age-dependent nondisjunction: Molecular studies of trisomy 16. Am J Hum Genet 57:867-874.

Hattori M (2000) The DNA sequence of human chromosome 21. The chromosome 21 mapping and sequencing consortium. Nature 405:311-319.

Holschneider AM, Meire-Ruge W, Ure BM (1994) Hirschsprung·s disease and allied disorders. Eur J Pediatr Surg 4:260-266.

Klein RM (1989) Small intestinal cell proliferation during development. In: Lebenthal E, ed., Human gastrointestinal development. New York Raven Press, pp. 367-392.

Lasek RJ, Oblinger MM, Drake PF (1983) The molecular biology of neuronal geometry; the neurofilament genes influences axonal diameter. Cold Spring Harb Symp Quant Biol 48:431-444.

Li JC, Busch LC, Kuhnel W (2000) Immunohistochemical study on gastroenteric nervous system in trisomy 16 mice: an animal model of Down syndrome. World J Gastroenterol 6:793-799.

Pletcher MT, Wiltshire T, Cabin DE, Villanueva M, Reeves RH (2001) Use of comparative physical and sequence mapping to annotate mouse chromosome 16 and human chromosome 21. Genomics 74:45-54.

Quinn FMJ, Surana R, Puri P (1994) The influence of trisomy 21 on outcome in children with Hirschsprung·s disease. J Pediatr Surg 29:781-783.

Román V, Krecsmarik M, Bagyánszki M, Fekete É (2001) Evaluation of the total number of myenteric neurons in the developing chicken gut using cuprolinic blue histochemical staining and neurofilament immunocytochemistry. Histochem Cell Biol 116:241-246.

Scheuermann DW, Stach W, Timmermans JP (1987) Topography, architecture and structure of the plexus submucosus internus (Meissner) of the porcin small intestine. Acta Anat 129:96-104.

Stach W (1989) A revised morphological classification of neurons in the enteric nervous system. In: Nerves and the gastrointestinal tract. Singer MV and Goebell H (eds). Kluwer Academic Publishers. Lancester p. 29.

Timmermans J-P, Hens J, Adriaensen D (2001) Outer submucous plexus: An intrinsic nerve network involved in both secretory and motility processes in the intestine of large mammals and humans. Anat Rec 262:71-78.

Yanagihara J, Shimotake T, Deguchi E, Iwai N (1992) Histological investigation of the myenteric plexus of the entire gut in an infant with hypogenesis of the intestine. Eur J Pediatr Surg 2:114-117.

Weaver LT, Austin S, Cole TJ (1991) Small intestinal length: a factor essential for gut adaptation. Gut 32:1321-1323.