EMBRYOLOGY OF NEUROCUTANEOUS SYNDROMES

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Introduction

The neurocutaneous diseases and neurocutaneous syndromes are a broad group of congenital disorders with diverse genetic, clinical and pathological features that share in common developmental lesions of the skin and of the central and peripheral nervous systems. Subcutaneous and systemic involvement is common. In many of these conditions another feature is a tendency to develop tumors in multiple sites of the body. Many of these disorders are hamartomatous in nature, and produce benign tumors, but patients also may develop malignant tumors. The etiology has been identified in several conditions: such cases are properly referred to as *neurocutaneous diseases*. In the case of neurofibromatosis and tuberous sclerosis complex, neoplasias can be explained because the genes responsible for the disease also are tumor-suppressor genes. Many neurocutaneous syndromes manifest overgrowth in one area, region or one side of the body, usually progressive (Cohen et al. 2001). Asymmetry and hemimegalencephaly are common features in several neurocutaneous syndromes (Flores-Sarnat 2002). The clinical features may be present at birth or become manifest later.

One of the constant and intrinsic features of the neurocutaneous syndromes, the skin lesions (mainly in the form of flat spots), occur in a big variety of shapes (round, oval, ash leaf, lines, whorls, etc.), sizes and number. There is little variation in color: they are hypo- or hyperpigmented, or red if vascular lesions. These spots may appear in any site on the body. In particular cases the hair, iris and meninges also may be affected. Abnormalities in vascular and adipose tissue, in the form of angiomas or lipomas, are a common feature.

Initially this group of disorders was named "phakomatosis" and consisted of only three entities: tuberous sclerosis, neurofibromatosis, and von Hippel-Lindau disease. Over time, more than 50 disorders have been added to this category, some without a developmental basis; the list continues to grow. It is, therefore, important to distinguish two categories of neurocutaneous syndromes as *primary* and *secondary* because of their different origins and prognoses.

Traditionally, it was considered that the common origin of skin and central nervous system from ectoderm explained the pathogenesis of the neurocutaneous syndromes. However, it was soon noted that mesodermal and endodermal tissues also were involved. Furthermore, with the advent of molecular genetics, the traditional concept of three germ layers has been challenged because the expression of many developmental genes is not restricted to one germinal layer. At present, there are many clues, both clinical and molecular, that support the new concept that an abnormality in the formation, migration or differentiation of neural crest cells is the common pathogenesis for most, if not all, *primary* neurocutaneous syndromes.

Many derivatives of the neural crest are involved in the neurocutaneous syndromes, particularly melanocytes that explain most of the skin lesions. Another clue is the abnormal pattern of migration of the melanocytes in several neurocutaneous syndromes that follow the lines of Blaschko, as observed in hypomelanosis of Ito and incontinentia pigmenti.

Neural crest cells are first recognized shortly after gastrulation, though they are not committed to their diverse lineages until later. After dorsal closure of the neural tube, neural crest cells separate and migrate throughout the embryo to form many structures of ectodermal origin (e.g. dorsal root and autonomic ganglia, peripheral nerve sheaths) and mesodermal origin (e.g. blood vessels,

melanocytes, adipose tissue, membranous bone, connective tissue, most of ocular globe). Terminal differentiation occurs after migration is complete. Three regions of the neural tube generate neural crest: rhombencephalon, mesencephalon and prosencephalon, each with a different migratory pattern. Some of the most important genes promoting neural crest differentiation and migration are those with a dorsalizing influence in the vertical axis of the neural tube (e.g. *PAX3*, *BMP4*, *ZIC2*), some segmentation genes (e.g. *WNT1*), genes that inhibit neural crest (e.g. *EGR2*) and neural crest-specific differentiating genes (e.g. *SLUG*, *SOX10*). In the neurocutaneous syndromes, diverse features may result from abnormal neural crest differentiation, providing a more encompassing embryological basis for these disorders than the traditional view that these syndromes are somehow related to skin and brain because both are ectodermal derivatives. Abnormal angiogenesis, regions of abnormal pigmentation, nerve sheath proliferations, lipomas and disorders of chromaffin tissue are frequent features. Interactions between genes associated with these disorders and others essential to neural crest formation, migration and differentiation, are a likely molecular genetic basis for these diseases. The craniofacial abnormalities associated with many neurocutaneous syndromes and the characteristic skin lesions emphasize an important inductive role of the neural tube upon the development of non-neural tissues, mediated through neural crest.

The classification of neurocristopathies can now be expanded to include many neurocutaneous syndromes. On the other hand, known neurocristopathies such as multiple endocrine neoplasia type II and familial medullary thyroid carcinoma are now included in the group of neurocutaneous syndromes. Waardenburg syndrome, recognized as a typical neurocristopathy, also can be considered a neurocutaneous syndrome.

Historical perspective and terminology

Though many diseases we now identify as neurocutaneous syndromes were described in the 19th century, the association of brain and skin developmental abnormalities was first made in 1920 by Van der Hoeve, a Dutch ophthalmologist, who observed similar retinal lesions between tuberous sclerosis, neurofibromatosis and von Hippel-Lindau disease. He coined the term *phakoma* and the concept of *phakomatosis* (*phakos* Greek = lentil, spot; lens-shaped), to describe the disseminated lentiform retinal lesions observed in that group of hereditary disorders (Van der Hoeve 1920, 1932). The term became inappropriate when Van der Hoeve included Sturge-Weber syndrome, which is not associated with phakomas or hamartomas. Etymologically it also is inadequate because it does not include the nervous system. It continues to be used by some contemporary authors, in a less broad context. In any case, its use should be reserved to those conditions which manifest retinal hamartomas ("phakomas"), corresponding to the original description.

The term *neurocutaneous syndromes* was introduced by Yakovlev and Guthrie in 1931 to describe "congenital malformations affecting more or less electively the ectodermal structures, i.e., the nervous system, the skin, the retina, the eyeball and its contents; sometimes visceral organs are also involved" (Yakovlev and Guthrie 1931). In their review they cited neurofibromatosis 1, tuberous sclerosis and Sturge-Weber syndrome. At that time the neurocutaneous syndromes were considered to originate from ectoderm, even though they recognized that the vascular anomalies in Sturge-Weber syndrome were mesodermal derivatives. However, the term "neurocutaneous" also is technically incorrect because *cutaneous* denotes only the epidermis and dermis, but subcutaneous lesions such as lipomas and subcutaneous neurofibromas also frequently appear in neurocutaneous syndromes. Moreover, it is now known that these syndromes involve not only ectodermal derivatives, but many structures other than brain and skin. Another problem is that *neurocutaneous* is a nonspecific term which might include any condition that affects the skin and nervous system, without being developmental in nature. It has the merit of linking the two most obvious manifestations of this group of disorders. Because of its long established and widespread usage, the introduction

of another nomenclature at this time would impede, rather than enhance, scientific communication.

Hamartoma refers to any abnormal growth that is made up of tissue composed of disorganized cells with dysplastic cytoarchitecture and situated within its organ of origin. A hamartoma occurs when a tissue does not develop completely or has ambiguous or mixed cellular lineage. Hamartomas can occur throughout the body, in any tissue.

It is important to make a distinction between *primary* and *secondary* neurocutaneous syndromes because, as mentioned previously, they have different pathogeneses and prognosis and the approach to management also is different. Primary neurocutaneous syndromes are developmental, dysgenetic conditions. Secondary neurocutaneous syndromes are not developmental disorders; they are the result of, or complications of, previous conditions, usually metabolic diseases. Examples include: Fabry disease, a lysosomal storage disease caused by deficiency of a-galactosidase; Lesch-Nyhan disease, due to a disorder in purine metabolism, exhibits cutaneous lesions secondary to accidental or self-inflicted injuries; Menkes disease is secondary to abnormal copper transport and metabolism; the cutaneous and central nervous system lesions are secondary to the metabolic defect.

Many neurocutaneous syndromes are now really diseases. A *syndrome* is a constellation of symptoms and signs, of unknown etiology, shared by a group of patients. When the etiology is discovered, whether it be infectious, metabolic or genetic, the syndrome is promoted in status to a *disease*.

Pathogenesis and molecular genetics

Throughout most of the 20th century, clinicians and pathologists tried to discover a common pathogenetic theme in the group of neurocutaneous syndromes, but this approach has been difficult because each disease varies greatly from the others in clinical presentation, genetics, pathological findings and imaging characteristics. Despite the traditional view is that they are diseases of ectoderm, even early 20th century investigators were troubled by the fact that many, if not all primary neurocutaneous syndromes, also involved tissues of mesodermal or endodermal origin. Tuberous sclerosis complex was the prototype of multisystemic involvement most frequently cited, with hamartomas not only of the brain and skin (ectoderm), but also of the heart and kidneys (mesoderm) and of endocrine glands and liver (endoderm) (Gómez et al. 1999, Curatolo 2003). Even some cutaneous lesions, such as the facial angiofibromas (inappropriately named "adenoma sebaceum" because they are neither adenomatous nor sebaceous), are of mesodermal origin. Angiomas are features common to many neurocutaneous syndromes, and also are mesodermal (Roach and Miller 2004, Santos et al. 2004). In our attempt to understand the embryology of neurocutaneous syndromes, we found the traditional theory of three germ layers unsatisfactory. The search for a common pathogenesis led us to conclude that the neural crest was the common thread in all of these syndromes.

With the advent of molecular genetics in the late 1980s, it soon became apparent that genetic expression does not restrict itself to the artificially assigned boundaries of the classical, time-honored "germ layers" and that the same gene families and individual genes contribute to developmental programming in multiple tissues. The entire traditional concept of germ layers is now under scrutiny and revision by contemporary embryologists. It may, in the future, become a concept cited only as an historical footnote. With it, the explanation that the neurocutaneous syndromes have a common link because brain and skin are both ectodermal derivatives no longer is tenuous as a rational basis.

Embryology of neural crest tissue

The first description of the neural crest was done by the Swiss/German anatomist-embryologist His (1868); however, it took almost a century for this structure to attract the attention of clinical investigators (Pages 1955, Small 1955).

Neural crest cells are first recognized at the lateral margin of the neural placode shortly after gastrulation, though they are not committed to their diverse fates until later. The neural crest is a transient population of embryonic cells derived from ectoderm

and defined by their migratory behavior and ability to form numerous derivatives (Basch et al. 2000). As neurulation proceeds, the curling of the neural placode results in these cells becoming dorsomedial in the neural groove. With closure of the dorsal midline to form the neural tube, the neural crest cells separate and begin migrating along prescribed routes throughout the embryo rather than remaining confined to the neural tube, initiating the formation of the peripheral nervous system, including dorsal root and autonomic ganglia, Schwann cells of peripheral nerves and chromaffin tissue in the adrenal medulla, carotid body and other sites. Neural crest also differentiates as cells traditionally regarded as mesodermal in origin, including melanocytes, endothelial cells, smooth muscle of blood vessels, interstitial connective tissue, the cranial meninges, the sclera of the eye, cartilage and membranous (but not endochondral) bone, especially the craniofacial skeleton (Tan and Morriss-Kay 1985, Le Douarin and Kalcheim 1999, Basch et al. 2000). When neural crest meets an epithelium, cartilage forms; when it meets mesodermal tissue, membranous bone forms. This explains why we have cartilage in our ears and bone in our orbits. Neural crest cells terminally differentiate into their diverse lineages only after reaching their final destination.

Neural crest is so pervasive that some authors have suggested its status be promoted as a fourth germ layer (Le Douarin and Kalcheim 1999, Hall 1999) but we disagree. Though incipient neural crest cells first appear at the lateral margins of the neuroepithelial placode on the day of gastrulation, these cells are not irreversible "committed" to a specific fate. Lineage analyses demonstrate that individual neural fold cells can form epidermis or neural crest cells, hence they are not truly neural crest until after gastrulation. Moreover, to create a fourth germ layer, other germ layers would have to yield tissues previously classified as derived from ectoderm and mesoderm in particular. But since the whole concept of embryonic germ layers may, in future, be regarded as an obsolete concept from the pre-molecular genetic era, this point is of little importance.

The development of neural crest derivatives can be divided into stages: a) specification of cells at the lateral borders of the neural plate as potential neural crest precursors; b) commitment of these cells at the time of closure of the neural tube as neural crest precursors, situated adjacent to the midline in the dorsomedial part of the closed neural tube; c) delamination or separation of these cells from the neural tube; d) migration into the periphery of the body, but not crossing the midline except in unpaired structures such as the heart and intestine; e) terminal differentiation as specific types of cells, or cellular lineage and diversification, after reaching the final position in the body. All of these stages are genetically programmed or influenced by genetic factors in surrounding tissues (Baker 2005). A cell is not formally regarded as neural crest until after it "delaminates" or separates from the neural tube; failure to delaminate and migrate results in further differentiation of neural crest precursor cells within the neural tube (Borchers et al. 2001).

Neural crest arises segmentally in all three primitive cerebral vesicles: rhombencephalon, mesencephalon and prosencephalon. Neural crest cells migrate in a somewhat different manner from each part of the embryonic neural tube after segmentation and the formation of neuromeres at 4–8 weeks gestation. The neural crest may be divided into three groups on this basis. The prosencephalic neural crest migrates rostrally into the head as a midline *vertical sheet* of cells (Puelles and Rubinstein 2003). The mesencephalic neural crest, which arises not only from the mesencephalic neuromere (i.e. r0, future midbrain), but also from the first two hindbrain rhombomeres (neuromeres r1 and the rostral half of r2), migrates as *streams* of cells (Fig. 1). The rhombencephalic neural crest, arising from the hindbrain (caudal half of r2 through r7 and also r8, which forms the spinal cord), migrates as *segmental blocks* of cells (Bronner-Fraser 1994, Carstens 2004).

Lists of structures derived from the neural crest are traditionally arranged according to anatomical zones and histological categories (Jambart et al. 1979; Jones 1990; Bolande 1974, 1997). We are modifing these lists to provide an embryological approach, with the current terminology (Table 1). The next stage awaits the discovery of specific genes involved in the development of each of these structures.

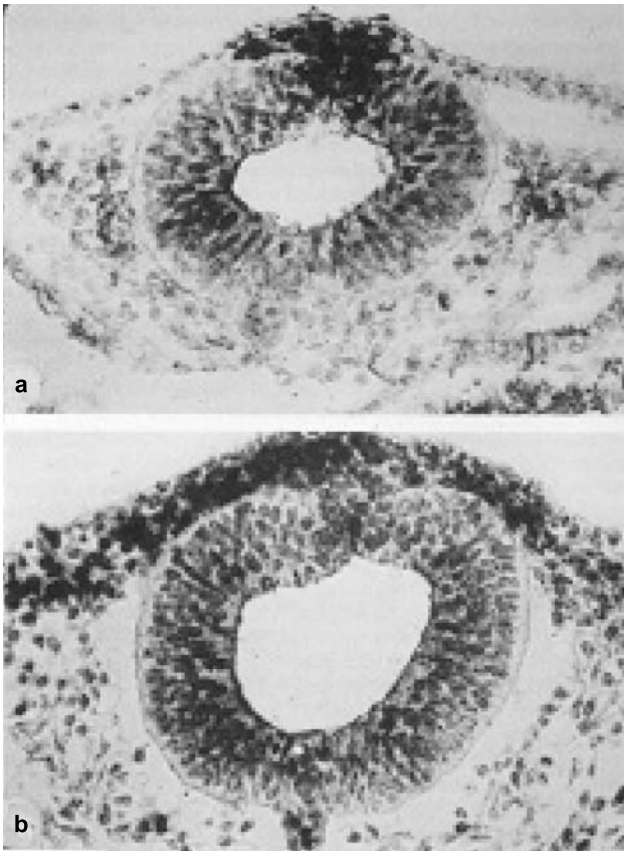

Fig. 1. Transverse sections of mesencephalic rhombomere (midbrain) in chick embryo. (**a**) Immediately after neural tube closure at the midbrain, neural crest cells (marked in black) are seen dorsomedially next to the midline. (**b**) A few hours later, these same cells are seen migrating away from the neural tube into the periphery as a stream, where they will proceed rostrally to form many structures of the face and cranium. A defect in the dorsal part of the neural tube at this level can interfere with mesencephalic neural crest formation and/or migration, resulting in midfacial hypoplasia. Reproduced with permission from: Le Douarin N and Kalcheim C (1999) The Neural Crest, 2nd ed. Cambridge: Cambridge University Press.

Neural crest is responsible for much of craniofacial development, including membranous bones of the face and cranial vault, though not the cranial base. In the face, mesencephalic neural crest forms not only structures of the peripheral nervous system, such as the ciliary ganglion and Schwann cells of nerves, but also many non-neural tissues of mesodermal origin: melanocytes of the skin and iris, stria vascularis of the cochlea, most of the globe of the eye including the sclera and cornea; connective tissues and vascular structures (Bronner-Fraser 1994, Le Douarin and Kalcheim 1999, Hall 1999).

Though patterns of genetic expression in the hindbrain contribute to the segmental arrangement of neural crest cells, cellular migratory pathways also are guided by attractant and repulsant paracrine molecules secreted by surrounding tissues such as the otic capsule, the somites, and the vertebral neural arches. In addition, neural crest cells possess *integrin* receptors for interacting with extracellular matrix molecules (Bronner-Fraser 1994). Changes in the distribution of *extracellular matrix* components during neural crest migration impose migratory guidance limits as well (Sadaghiani et al. 1994). The extracellular matrix glycoprotein *tenascin* is required for proper cranial neural crest migration and its absence in the chick embryo leads to neural tube defects and aggregates of ectopic neural crest cells (Bronner-Fraser 1988). Other extracellular matrix molecules that promote neural crest migration include fibronectin, laminin and collagen types I, IV and VI (Perris and Perissinotto 2000). Ephrins, by contrast, repulse neural crest divert caudally (Kalcheim 2000, Krull 2001, Baker 2005).

Protein kinase C is an inhibitory factor on neural crest at various stages. If this protein is inhibited in mouse embryos at the neural tube stage and in cell cultures, neural crest precursor cells precociously delaminate and migrate away from the neural tube before their programmed time (Newgreen and Minichiello 1995, Rathjen et al. 2002). *Sox10*, associated with later differentiation of neural crest cells (see below), also is upregulated by protein kinase C inhibition (Rathjen et al. 2002). The phase of the mitotic cell cycle, in particular the transition from G1 to S-phase, is another important determinant of delamination; blocking this transition also impedes delamination of the neural crest (Burstyn-Cohen and Kalcheim 2002, Baker 2005).

The neural crest consists of a series of overlapping cell populations that thus differ in their migratory pathways and fates. Why neural crest precursors are so heterogeneous, why neural crest stem cells exist with multiple potentials, and even whether stem

cells arising from the neural tube are joined by surrounding cells from the mesodermal germ layer are not as well understood as are their migratory pathways outside the neural tube (Selleck et al. 1993). As with other parts of the neural tube, neural crest tissue has a rostrocaudal gradient of differentiation. The fate of neural crest cells is not entirely predetermined; environmental factors may induce differentiation as other cells than were originally intended. For example, although early-migrating neural crest cells generally form dorsal root ganglion cells, when these early-migrating cells are ablated, the late-migrating neural crest cells that ordinarily form mesodermal structures change their fate to become

Table 1. Embryonic distribution of neural crest derivatives

Prosencephalic Neural Crest
Paramedian frontal bones around metopic suture
Melanocytes of skin at frontal midline, extending onto midline of nose
Melanocytes of hair follicles of rostral frontal midline scalp ("forelock")
Subcutaneous connective tissue, including adipocytes, microvasculature of frontal midline and Schwann cells of small cutaneous nerves
Mesencephalic Neural Crest
Membranous bones of face including orbit, otic capsule, sphenoid
Membranous bones of cranial vault including rostral 2/3 of parietal bone
Cartilage of face, including nasal root and ears
Hard and soft palate
Trigeminal neurovascular bundles (including ganglia, Schwann cells and vessels)
Stria vascularis of cochlea
Ciliary ganglion
Parasympathetic nerves (except axons) to face and iris
Connective tissue, including adipocytes, and vasculature of face and cranium, including smooth muscle of arterioles and venules, part of dermis
Connective tissue of adenohypophysis (anterior pituitary)
Cranial leptomeninges and dura mater (not spinal meninges or tentorium cerebelli)
Most of ocular globe (except retina, choroid, cornea)
Odontoblasts
Melanocytes of iris, skin of face and most of scalp hair
Rhombencephalic Neural Crest (includes spinal cord)
Facial neurovascular bundle
Glossopharyngeal neurovascular bundle
Vagal neurovascular bundles
Spinal nerve roots and dorsal root ganglia (except neurons and axons)
Sympathetic nerves and ganglia (except axons), including those of face, paravertebral sympathetic chain, superior cervical ganglion, periaortic complexes, celiac ganglion, mesenteric nerves
Adrenal medulla
Carotid body and other chemoreceptors
Parasympathetic nerves and ganglia (except axons) including submucosal and intramuscular plexus of intestine and to lungs, dermal and endocrine glands, pelvic plexus
Melanocytes of skin and hair of posterior 1/3 of scalp, neck and body (trunk and extremities)
Cartilage of pharyngeal arches and part of chondrocranium (not cranial base or basioccipital, exoccipital or supraoccipital bones)
Connective tissue of lingual, salivary buccal, parathyroid glands and thymus; adipocytes
Thyroid C-cells

neurons. Nevertheless, transplantation of early-migrating neural crest cells does not result in production of neurons under all conditions (Raible and Eisen 1996).

Neurotrophic factors, such as neurotrophin-3 (NT-3), also influence the fate of neural crest cells and are essential for survival of sympathetic neuroblasts and innervation of specific organs (El Shamy et al. 1996). NT3 is the principal and perhaps the only neurotrophin needed by neurons of the myenteric plexus (Gershon 1999; Chalazonitis 1996, 2004), but other neural crest derivatives require other factors. Gene products of *bone morphogenic proteins* (*BMP2* and *BMP4*) regulate the onset of NT3 during fetal gut development, and *BMP4* and NT3 (with its receptor TrkC) are needed to preserve the integrity of the submucosal and myenteric plexuses. Nerve growth factor (NGF), the first neurotrophin identified, was first demonstrated in dorsal root ganglia. Brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF) and glial-derived neurotrophic factor (GNTF) all are associated with neural crest migration or differentiation (Sieber-Blum 1999).

The origin of neural crest is topographically unequal in the neuraxis. The streams of cells arising in the midbrain contribute to craniofacial structure. In the hindbrain, migratory mesencephalic neural crest cells from r1 and r2 populate the trigeminal ganglion and streams of rhombencephalic neural crest form the mandibular arch; cells from r4 form the hyoid arch, the geniculate and vestibular and cochlear ganglia; those from r6 populate the third and fourth pharyngeal arches and associated peripheral ganglia (Bronner-Fraser 1994, 1995). Rhombomeres 3 and 5 do not appear to have neural crest cells, but actually neural crest cells are generated in r3 and r5 but they fuse rostrally and caudally with neural crest cells of adjacent rhombomeres to migrate together, because of the EGR2 gene expressed only in r3 and r5 as discussed below (Bronner-Fraser 1994). This also explains the fusion of the proximal maxillary and mandibular branches of the trigeminal nerve.

In phylogenetic evolution, neural crest tissue is unique in vertebrates and absent in invertebrates. Some protochordates, such as amphioxus, express some genes that also are expressed in vertebrate neural crest, but these are not detected either in the lateral border of the neural plate during development or in the dorsal "neural tube" in the adult (Meulemans et al. 2003). The central nervous system of the mature amphioxus remains in a stage resembling the neural groove of vertebrates, however, because the dorsal midline does not fuse to form a complete neural tube and this is the region where neural crest originates in vertebrates; the central canal in amphioxus is a vertical slit lined by ependyma but without a roof plate and open at the dorsal surface (Sarnat and Netsky 1981).

Genetic programming of neural crest

Many genes are involved in the formation and migration of neural crest and, finally, the terminal differentiation into the various types of cells it forms. Neural crest cells do not differentiate until they arrive in their target zone. The recent explosion of molecular genetic data diminishes the importance of embryonic germ layers, long believed to be fundamental in cellular lineage, because it is now recognized that the same organizer and regulator genes program development and cytological differentiation in various tissues, and that genes do not restrict their expression to individual embryonic germ layers. Examples are the *HOX* and *PAX* families, both primordial in the embryonic segmentation of the neural tube, but also in the ontogenesis of bone, kidney, gut and many other tissues. Even single gene deletions or mutations may downregulate other downstream genes in a cascade, permit the overexpression of antagonistic genes or otherwise alter complex multigenetic interactions. Trophic factors important in neural crest migration and maturation also might be defective in some of these syndromes. Neural crest induction occurs continuously over a long period starting at gastrulation and persisting well past the time of neural tube closure [4]. Neural crest can be induced to form in early neuroectoderm by the proximity of non-neural surface ectoderm (Lumsden et al. 1991, García-Castro and Bronner-Fraser 1999). Exposure of the most rostral neuroectoderm at its boundary with epidermis in *Xenopus*, to *BMP* plus

bFGF, *Wnt8* or retinoic acid, transforms this tissue into neural crest (Villanueva et al. 2002). The lateral border of the primitive neural plate, where neural crest precursors first develop, is specified by the activity of *BMP* and *Dlx* transcription products, but these molecules do not specify neural crest cells (Villanueva et al. 2002, McLarren et al. 2003, Woda et al. 2003).

Many genes are essential to the formation of neural crest, but the most important are those having a strong dorsalizing effect in the vertical axis of the neural tube: *ZIC2*, *BMP4*, *BMP7*, *PAX3*. The transforming growth factor-beta (TGFβ) superfamily, and in particular *BMP4* and *BMP7*, promote neural crest differentiation at the time of neural tube closure; these two genes can even substitute for non-neural ectoderm in inducing neural crest cells (Liem et al. 1995). Ventralizing genes of the vertical axis, such as *SHH*, produced by both notochord and floor plate cells of the neural tube, inhibit neural crest formation (Bronner-Fraser 1995). Experimentally, either notochordal tissue or *Shh*-expressing cells grafted adjacent to the neural folds prevent neural crest formation (Selleck et al. 1998). The gene *Noggin*, a strong antagonist of *BMP* genes, also inhibits neural crest formation (Dickinson et al. 1995).

Delamination of neural crest precursor cells from the dorsal neural tube to the periphery is mediated or regulated by several genes, that include *FoxD2*, *RhoB* (activated by *BMP4*), and *Slug* in particular (Nieto et al. 1994, Cano et al. 2000, Dottori et al. 2001, Baker 2005).

At a later stage in neural tube development, the segmentation genes that program the formation of neuromeres also can promote neural crest, especially those with a dorsalizing effect in the vertical axis. The segmentation homebox *Wingless* family, particularly *Wnt1* and *Wnt3a*, not only are important for the formation of neuromeric compartments and their boundaries in the hindbrain, but also promote neural crest formation (Dickinson et al. 1995, LaBonne 2002). The human gene *EGR2* (known as *Krox-20* in the mouse) is another segmentation homeobox gene, but expressed only in rhombomeres r3 and r5. *EGR2* inhibits neural crest formation, but the incipient neural crest cells of these two rhombomeres shift to adjacent rhombomeres where *EGR2* is not expressed, and mix with the neural crest being generated in those rhombomeres, so that neural crest cells that migrate caudally around the otic vesicle are from both r5 and r6 (Bronner-Fraser 1994). *Hox-1.5* and *Hox-2.9* regulate the premigratory and migratory neural crest cells from r4 (Chisaka and Capecchi 1991, Hunt et al. 1991). *Hox* family genes encode the posterior part of the brain, rhombencephalic neural crest and the pharyngeal arches, whereas this programming function in the rostral brain, including the mesencephalic and prosencephalic neural crest and viscerocranium (cranial vault) is correspondingly regulated by the gene *Otx2* (Kuratani et al. 1997). Expression of *Otx2* is regulated by two "enhancers" with their caudal limit at the isthmus or mesencephalic-metancephalic boundary (Kurokawa et al. 2004). At least part of the migratory patterning is not as rigidly preprogrammed as previously thought, however, and the cranial neural crest may partly be a passive transfer of positional information from the brain to the periphery by not inhibiting, rather than actively promoting, cellular migration (Trainor and Krumlauf 2000).

The gene *SLUG* (*Snail* in invertebrates) seems to be essential for later stages of neural crest differentiation. Though it also is detected in early stages of the neural placode prior to neural crest migration and in early migratory cells to the periphery, its transcript is later is downregulated in later migration and also in vitro in the absence of tissue interactions (Basch et al. 2000, LaBonne and Bronner-Fraser 2000). *SLUG* then is later re-expressed in stronger form. *SLUG* is a "zinc-finger", defined as DNA-binding, gene-specific transcription factors consisting of 28 amino acid repeats with pairs of cysteine and histadine residues, each sequence folded around an ion of zinc (Nieto et al. 1994). In the amphibian embryo, *Slug* expression does not itself induce neural crest, but in the presence of *Wnt* signals. It yields a robust neural crest (LaBonne and Bronner-Fraser 2000). *BMP4* is upregulated in the isolated neural folds just prior to the expression of *Slug* (LaBonne and Bronner-Fraser 1998). *SOX10* is another gene involved in terminal neural crest differentiation, particularly in the rhombencephalic neural crest

migration to the gut and differentiation of ganglion cells for the submucosal and myenteric plexuses (Honore et al. 2003, Paratore et al. 2001).

Other genes implicated in neural crest development include *OTX* (*EMX1*,*2*), *PHOX*, *DLX*, *MASH1* and *TWIST*. The *PAX* and *MSX* families are of particular importance in craniofacial development associated with prosencephalic and mesencephalic neural crest migration (Bei et al. 2002). The proto-oncogene *c-myc* is another essential regulator of neural crest formation (Bellmeyer et al. 2003). Mutations of the RET proto-oncogene resulting in overexpression is associated with several neurocristopathies including multiple endocrine neoplasias type 2 and medullary carcinoma of the thyroid (Donis-Keller et al. 1993, Hofstra et al. 1994), neuroblastoma (Ikeda et al. 1990) and Hirschprung disease (Edery et al. 1994, Romeo et al. 1994).

Terminal differentiation of neural crest cells also is genetically regulated. The choice between differentiating as sensory (i.e. dorsal root ganglionic) neurons or autonomic ganglionic neurons depends upon exposure to *BMP2* expression in peripheral tissues, that probably emanates from the dorsal aorta; *BMP2* initiates *MASH1* expression, which leads to autonomic differentiation (Anderson 1997). Sensory neurons form in the absence of *BMP2*.

The concept of "neurocristopathies"

The term *neurocristopathy* was first introduced by Bolande in 1974 to denote a group of diverse diseases having a common origin in neural crest maldevelopment (Bolande 1974), with later updates (Bolande 1981, 1997). He divided the neural crest disorders into *simple* and *complex*; *dysgenetic* and *neoplastic*. A simple neurocristopathy is exemplified by aganglionic megacolon (Hirschsprung disease), in which segments of intestine lack submucosal and myenteric plexi of parasympathetic ganglion cells. Neurofibromatosis and neurocutaneous melanosis were cited by Bolande as examples of complex neurocristopathies, but he did not extend the concept to encompass all neurocutaneous syndromes. Bolande also noted that the complex diseases tended to follow Mendelian inheritance, and that simple neurocristopathies were usually sporadic.

In recent years, and particularly with the fountainhead of new molecular genetic data that continues to emerge, the importance of neural crest as an inducer not only of peripheral neural structures such as ganglia but of many tissues in craniofacial development and other peripheral mesodermal structures is becoming more and more evident.

We submit that many manifestations of the category traditionally been regarded as "primary neurocutaneous syndromes" may be attributed in large part to abnormal neural crest migration and differentiation as well, thus expanding Bolande's original concept of neurocristopathies to an entire category of abnormal neural tube induction of non-neural peripheral structures of the body that represent neural crest derivatives. Bolande's original and updated division of the neurocristopathies into "simple" and "complex" (Bolande 1974, 1997) has merit for convenience in clinical classification, but the two categories may blend when the genetic basis of all become known.

Relation between neural crest and neurocutaneous disorders

For the past three decades, neurofibromatosis and neurocutaneous melanosis were the only neurocutaneous syndromes consistently considered to be related to abnormal neural crest. A few authors have speculated on a possible role of neural crest in the pathogenesis of other neurocutaneous syndromes in isolated cases, but until now we have not found publications with the proposal that the embryological basis for the entire group of "primary" neurocutaneous diseases is defective neural crest tissue due to genetic mutations that impede or alter the formation, migration or terminal differentiation of neural crest cells. In the case of encephalocraniocutaneous lipomatosis or Haberland syndrome, a link with cephalic neural crest was suggested in 1989 (Bamforth et al. 1989), but only rarely cited in further publications (Jozwiak and Janniger 2005)

The primary neurocutaneous syndromes are here reexamined in this context, with a premise that

they are embryological disorders of neural crest tissue, hence can be considered neurocristopathies (Sarnat and Flores-Sarnat 2005).

A common theme amongst the various neurocutaneous syndromes, despite their diverse genetics and clinical presentation, is that all include many features explicable as defects in neural crest. Examples include the circumscribed cutaneous lesions with deficient or excessive melanin pigment, frequent vascular malformations in skin and in other organs and involvement of peripheral nerve sheaths. Even many lipomas associated with several neurocutaneous syndromes are terminal overgrowths due to dysregulation of neural crest. Some examples of proliferation of adipose tissue are: epidermal nevus syndromes, Proteus syndrome, Klippel-Trenaunay syndrome, encephalocraniocutaneous lipomatosis, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Delleman syndrome.

Another characteristic feature in several neurocutaneous syndromes is the pattern of skin lesions that follow Blaschko lines, described in detail by this author a century ago, who noted this pattern assumed by many different naevoid and acquired skin and mucosae diseases does not follow nerves, vessels, or lymphatics (Blaschko 1901). He observed that these lines not only did not correspond to any known anatomical basis, but were remarkably consistent both from patient to patient and even from one disease to another. He proposed an embryonic origin for these lines, but did not suggest a mechanism. Their cause remained unknown, but we now can attribute this unique abnormal pattern of linear cutaneous pigmentation to abnormal migration of neural crest cells in the skin.

The following are examples of well known neurocutaneous syndromes in the context of derivation from neural crest tissue. We include Waardenburg syndrome, because it easily fulfills the criteria of a primary neurocutaneous syndrome:

Neurofibromatosis 1: This autosomal dominant disease is the most frequent and clinically well described of the neurocutaneous syndromes (Pascual-Castroviejo 2001, Roach and Miller 2004, Santos et al. 2004)). Many features support the concept that its pathogenesis is indeed a result of defective neural crest: a) Café-au-lait and depigmented spots involve abnormal melanocyte differentiation, excessive or deficient, from rhombencephalic neural crest in particular. b) Neurofibromas (and also Schwannomas) of peripheral nerves, including small cutaneous and subcutaneous nerves, are benign nerve sheath growth disorders of neural crest origin. c) A high incidence of pheochromocytoma in the adrenal medulla is of neural crest origin. d) An increased incidence of hypertelorism is found in patients with neurofibromatosis 1, a minor dysmorphism indicating involvement of prosencephalic neural crest with incomplete formation of the intercanthal ligament, as described below. e) Nerve sheath tumors may be directly attributed to defective neural crest, but the *NF1* gene also is a tumor-suppressor gene and its impairment predisposes to neoplasia. It should be noted that the cranial meninges are of neural crest origin, but that the spinal meninges are derived from paraxial mesoderm (Table 1).

Optic nerve gliomas are common in this disease, but are not of neural crest origin; the defective tumor-suppressor function of the *NF1* gene is more likely the basis, because this effect is not limited to neural crest.

Tuberous sclerosis complex: This is the most complicated of the neurocutaneous syndromes and involves the largest number of tissues arising from all three germ layers of classical embryology (Gómez et al. 1999, Curatolo 2003). Some, but not all, lesions can be attributed to neural crest defects: a) Cutaneous lesions, particularly hypomelanotic macules, b) Shagreen patches, are due in part to abnormal segmental melanocytic distribution. c) The characteristic facial angiofibromas are derived from mesencephalic neural crest. d) Poliosis or hypopigmented scalp hair is another neural crest defect of melanocyte differentiation.

Hamartomas in the periventricular region and in the cerebral cortex ("tubers") are not neural crest derivatives. The two tuberous sclerosis genes also function as tumor-suppressor genes and this may promote the tumoural transformation of hamartomas in the periventricular region. One report has suggested that

a link between a primitive neuroectodermal tumor of bone and tuberous sclerosis may be a "maldevelopment of the neural crest or neurocristopathy" (Hindman et al. 1997).

Epidermal nevus syndromes: This umbrella term includes several disorders with different types of epidermal nevi and diverse systemic involvement. One of the best known epidermal lesions, the *linear sebaceous nevus of Jadasshohn*, is the prototype that initially drew our attention to the neural crest because of its characteristic features: a) This midline vertical linear pigmented, or occasionally depigmented, lesion of the forehead is a clear marker of prosencephalic neural crest distribution: the prosencephalic neural crest migrates as a vertical sheet in the midline of the head. b) The subcutaneous lipoma that affects the face asymmetrically (Egan et al. 2001, Flores-Sarnat 2002) is of neural crest origin. This lesion is often referred as "facial hemihypertrophy" or "facial hypertrophy" (Pavone et al. 1991, Zhang et al. 2003). Some patients develop a more severe, infiltrative lesion, called "congenital infiltrating lipomatosis of the face" (Slavin et al. 1983, Unal et al. 2000, Aydingöz 2002). c) The inflamatory linear verrocous epidermal nevus (ILVEN) has a particular pattern following lines of Blaschko that we consider of neural crest origin. d) Ocular (but not retinal) dysplasia is a common complication that may arise from neural crest.

Incontinentia pigmenti (Bloch-Sulzberger syndrome): a) The linear pigmented lesions on the trunk and extremities in this X-linked dominant disease are often said to correspond to "cleavage lines in the skin" (lines of Blaschko), but they are better explained as rhombencephalic neural crest migratory pathways to the dermis. b) Verrucous plaques and epidermal hyperplasia with hyperpigmentation, already observed at birth, indicate epidermal as well as dermal involvement of the same origin. c) Neovascularity and microangiomas in the eye and brain cause microinfarcts, the predisposing vascular lesions probably being of neural crest origin.

Hypomelanosis of Ito: a) The hypopigmented macules arranged over the body surface in sharply demarcated whorls, streaks and patches are present from birth (Pascual-Castroviejo et al. 1998), also following the lines of Blaschko that represent deficient melanocyte differentiation in rhombencephalic neural crest dermal territories. b) Dysfunction in sweating in some patients, with anhidrosis in the hypomelanotic areas, suggest abnormal eccrine gland cells, which derive from the neural crest (Steijlen et al. 2000). c) Neuroblastoma, a neoplasm derived from neural crest, has been reported in this syndrome (Oguma et al. 1996). Mental retardation, epilepsy and muscular hypotonia are present as evidence of CNS involvement.

Neurocutaneous melanosis: This rare syndrome was described in 1861 by Rokitansky and is characterized by the presence of giant or multiple congenital pigmented nevi, associated with benign or malignant melanocytic tumors of the CNS, particularly of the leptomeninges (Rokitansky 1861). The nevi are usually localized in the head, neck and dorsal spine, suggesting a rhombomeric distribution. Both melanocytes and cranial leptomeninges are neural crest derivatives. Neurocutaneous melanosis is frequently reported in association with the Dandy-Walker malformation (Cramer 1988, Chaloupka et al. 1996, Berker et al. 2000), but the common pathogenesis is not evident. Since Bolande first included this disorder as a neurocristopathy, other authors also have accepted the premise that neurocutaneous melanosis is a disorder of neural crest (Bolande 1974, 1981, 1997).

Sturge-Weber disease: The remarkable concordance of the cutaneous vascular lesions with branches of the trigeminal nerve has been observed for many years, with the inference that somehow the nerves are involved with the formation of capillary malformations of the face and scalp (Roach and Millar 2004). Fetal sensory nerves secrete a neurotrophic factor that serves as a potent stimulant of angiogenesis, accounting for arterial differentiation and the "neurovascular bundles" in the fetus (Mokouyama et al. 2002). An alternative explanation is that rather than the nerves causing the vascular malformation, both are in territories corresponding to neural crest migrations that form both nerve sheaths and small blood vessels. Many cases of Sturge-Weber disease

are more extensive, beyond the territories of the trigeminal nerve, with microcapillary malformations involving the neck, chest and back, but still within the distribution of rhombencephalic neural crest. Cutaneous lesions may be unilateral or bilateral. Epilepsy in this disease may result from microvascular malformations in the meninges, of neural crest origin, or in the brain. Gómez pointed out that Sturge-Weber syndrome is neither phakomatosis nor hamarmatosis (Gómez et al. 1999).

Klippel–Trenaunay syndrome, Klippel–Trenaunay-Weber syndrome: These terms are sometimes used interchangeably; however, Cohen et al. (2001) distinguishes between the syndrome described by Klippel and Trenaunay (1900) and that described by Parkes Weber (1907). Even when they are similar conditions, usually sporadic and characterized by developmental vascular abnormalities, there are some important clinical differences. The classical triad of Klippel–Trenaunay syndrome consists of: a) vascular malformations of the capillary, venous and lymphatic vessels; b) varicosities of unusual distribution, particularly the lateral venous anomaly; and c) unilateral limb enlargement, usually the lower extremity. In Parkes Weber syndrome, the arteriovenous fistulas, are the predominating feature. The cutaneous vascular lesions that characterize these syndromes derive from cells of neural crest origin.

Waardenburg syndrome (WS): This condition was first reported by Van der Hoeve in 1916 in 3 patients (Van der Hoeve 1916). Waardenburg added more cases and defined the main features of this condition (Waardenburg 1948, 1951). Waardenburg syndrome was amongst the first genetic diseases to be associated with neural crest (Ommen and McKusick 1979). Waardenburg syndrome is classified into four types: WS type I (WS1), WS type II (WS2), WS type III (WS3), and WS type IV (WS4). Though not previously cited as neurocutaneous syndromes, both cutaneous (hair, skin) and neurological deficits (neurosensory hearing loss) are constant features, hence can be grouped under this rubric. WS types I and III are caused by mutations of *PAX3* gene. Waardenburg syndrome type III (*Klein-Waardenburg*) includes variable musculoskeletal anomalies, and occasionally meningomyelocele and mental retardation. All of the characteristic facial features observed in Waardenburg type I that together present a pleasant feline facies (Flores-Sarnat 2007) can be explained by neural crest defects: a) The hypopigmentation of hair (typical white forelock of hair adjacent to the midline in the scalp) is due to lack of hair follicle melanocytes in the distribution of prosencephalic neural crest. b) Hypopigmented irides, sometimes asymmetrical (heterochromia), are due to a paucity of melanocytes in the iris. The reason for the asymmetry in some cases is uncertain, but may involve interactions with genes of bilateral symmetry. c) Lateral displacement of the medial canthi of the eyes (dystopia canthorum). d) The tubular nose due to hypoplasia of the alae nasi with a hyperplastic,

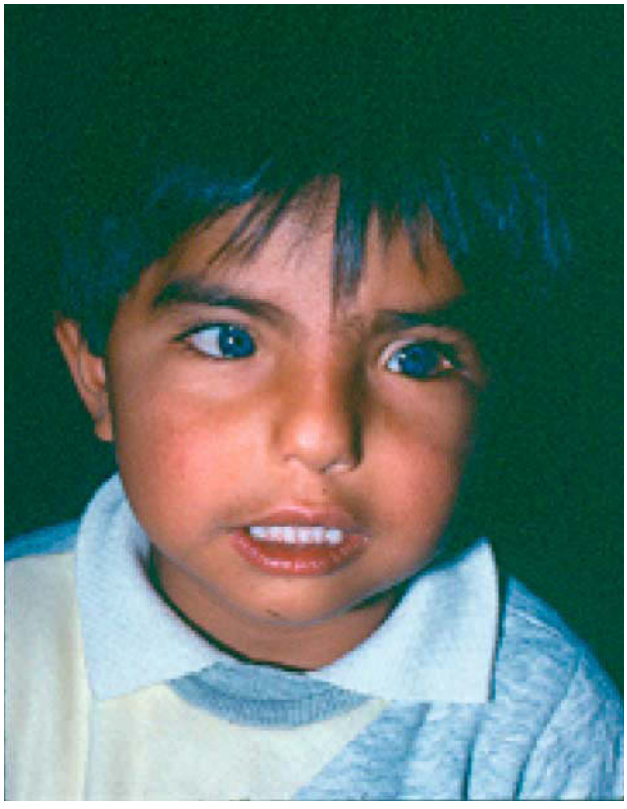

Fig. 2. Five-year-old boy with Waardenburg syndrome (WS) type I, showing typical features: sapphire blue eyes with dystopia canthi, hypertelorism, high nasal root with tubular nose and a small white forelock. He also has profound bilateral neurosensory deafness. His mother also has WS type I.

broad and high nasal bridge, suggests a defect of prosencephalic neural crest, probably with defective formation of the intercanthal ligament. e) A square jaw is explained by the derivation of membranous bone of the lower jaw from neural crest (Fig. 2). The neurosensory hearing loss is also the result of involvement of the cochlear *stria vascularis*, another neural crest derivative, secondarily causing degeneration of the cochlear hair cells and the auditory nerve.

WS type II was recognized by Arias and distinguished from WS1 for the lack of dystopia canthorum (Arias 1971). This is a melanocyte-specific disorder also characterized by deafness and hypopigmentation because of lack of melanocytes in the inner ear and skin, but without a distinctive face as in WS type I. It is due to a mutation in the *MITF* gene, essential for melanocyte differentiation. Recently, in two unrelated patients with WS2, homozygous deletions in SLUG were demonstrated (Sánchez-Martín et al. 2002).

Waardenburg type IV (*Shah-Waardenburg*) includes aganglionic megacolon (Hirschsprung disease) (Ommen and McKusick 1979), a defect of rhombencephalic neural crest; many of these patients have defective expression of *SOX10* during fetal development (Touraine et al. 2000, Chan et al. 2003), an essential gene for neural crest migration to the gut (Paratore et al. 2001). Mutations of the *SOX10* gene also are involved in some patients with Waardenburg type IV who additionally have congenital hypomyelinating neuropathy (Inoue et al. 2002), incorporating a defect in Schwann cell function, yet another cell of neural crest origin.

Because the genetic bases of the four forms are known, Waardenburg syndrome is ready to be elevated to the category of a disease and can be called "Waardenburg disease".

Reassessment of the classification of *neurocristopathies*

The classification of "neurocristopathies" can be expanded to include many primary neurocutaneous syndromes, in addition to neurofibromatosis and neurocutaneous melanosis. Waardenburg syndrome, on the other hand, is a recognized neurocristopathy, but not previously considered a neurocutaneous syndrome, though it easily fulfills the criteria. A better understanding of the primordial role of neural crest in the pathogenesis of the neurocutaneous syndromes may enable further subclassification of these diseases, based upon a common pathogenesis of abnormal neural crest induction of non-neural tissues. This category would supplement the scheme we previously proposed as an integrated morphological and molecular genetic classification of nervous system malformations (Sarnat 2000, Sarnat and Flores-Sarnat 2004). We also include Waardenburg syndrome as a neurocutaneous syndrome for the same reason that unifies the other diseases in this category.

This new concept of the embryology of neurocutaneous syndromes deriving from disturbances of neural crest formation or migration may lead to a reclassification of *primary* neurocutaneous syndromes as a category of neurocristopathies. Ultimately, the classification of the neurocristopathies and of the neurocutaneous syndromes will rest upon the identification of genetic mutations and interrelations with embryonic neural crest. How defective genes in the neurocutaneous syndromes interact with the many genes, including some that program symmetry, with transcription factors and with neurotrophic factors during normal neural crest development is largely unknown, but offers the promise of insight into the pathogenesis of these diseases.

References

Anderson DJ (1997) Cellular and molecular biology of neural crest cell lineage determination. Trends Genet 13: 276–280.

Arias S (1971) Genetic heterogeneity in the Waardenburg syndrome. Birth Defects 7: 87–101.

Aydingöz U, Emir S, Karh-Oguz K, Kose G, Buyukpamukcu M (2002) Congenital infiltrating lipomatosis of the face with ipsilateral hemimegalencephaly. Pediatr Radiol 32: 106–109.

Baker C (2005) Neural crest and cranial ectodermal placodes. In: Developmental Neurobiology (Rao MS, Jacobson M, eds.), 4th ed., pp. 67–127. New York: Kluwer Academic/Plenum Publishers.

Bamforth JS, Riccardi VM, Thisen P, Chitayat D, Friedman JM, Caruthers J, Hall BK (1989) Encephalocraniocutaneous lipomatosis. Report of two cases and a review of the literature. Neurofibromatosis 2: 166–173.

Basch ML, Selleck MAJ, Bronner-Fraser M (2000) Timing and competence of neural crest formation. Devel Neurosci 22: 217–227.

Bei M, Peters H, Maas RL (2002) The role of *PAX* and *MSX* genes in craniofacial development. In: Craniofacial Surgery (Lin KY, Ogle RC, Jane JA, eds.), pp. 101–112. Philadelphia: WB Saunders.

Bellmeyer A, Krase J, Lindgren J, LaBonne C (2003) The protooncogene *c-myc* is an essential regulator of neural crest formation in *Xenopus*. Devel Cell 4: 827–839.

Berker M, Oruckaptan HH, Oge HK, Benli K (2000) Neurocutaneous melanosis associated with Dandy-Walker malformation. Pediatr Neurosurg 33: 270–273.

Blaschko (1901) Die Neven-verteilung in der Haut in ihrer Beziehung zu den Erkrankungen der Haut. Beilage zu den Verhandlungen der Deutschen Dermatologischen Gesellschaft VII Congress, Breslau.

Bolande RP (1974) The neurocristopathies. A unifying concept of disease arising in neural crest maldevelopment. Hum Pathol 5: 409–429.

Bolande RP (1981) Neurofibromatosis – the quintessential neurocristopathy: pathogenetic concepts and relationships. Adv Neurol 29: 67–75.

Bolande RP (1997) Neurocristopathy: its growth and development in 20 years. Pediatr Pathol Lab Med 17: 1–25.

Borchers A, David R, Wedlich D (2001) *Xenopus* cadherin-11 restrains cranial neural crest migration and influences neural crest specification. Development 128: 3049–3060.

Bronner-Fraser M (1988) Distribution and function of tenascin during cranial neural crest development in the chick. J Neurosci Res 21: 135–147.

Bronner-Fraser M (1994) Neural crest formation and migration in the developing embryo. FASEB J 8: 699–706.

Bronner-Fraser M (1995) Origins and developmental potencial of the neural crest. Exp Cell Res 218: 405–417.

Burstyn-Cohen T, Kalcheim C (2002) Association between the cell cycle and neural crest delamination through specific regulation of G1/S transition. Dev Cell 3: 383–395.

Cano A, Pérez-Moreno MA, Rodrigo I et al. (2000) The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol 2: 76–83.

Carstens MH (2004) Neural tube programming and craniofacial cleft formation. I. The neuromeric organization of the head and neck. Eur J Paediatr Neurol 8: 181–210.

Chalazonitis A (1996) Neurotrophin-3 as an essential signal for the developing nervous system. Mol Neurobiol 12: 39–53.

Chalazonitis A (2004) Neurotrophin-3 in the development of the enteric nervous system. Progr Brain Res 146: 243–263.

Chaloupka JC, Wolf RJ, Varma PK (1996) Neurocutaneous melanosis with the Dandy-Walker malformation: a possible rare pathoetiologic association. Neuroradiology 38: 486–489.

Chan KK, Wong CK, Lui VC et al. (2003) Analysis of *SOX10* mutations identified in Waardenburg-Hirschsprung patients: differential effects on target gene regulation. J Cell Biochem 90: 573–583.

Chisaka O, Capecchi MR (1991) Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene *Hox 1.5*. Nature 350: 473–479.

Cohen MM Jr, Neri G, Weksberg R (2001) Overgrowth Syndromes. Oxford University Press, New York.

Cramer SF (1988) The melanocytic differentiation pathway in congenital melanocytic nevi: theoretical considerations. Pediatr Pathol 8: 253–265.

Curatolo P (ed.) (2003) Tuberous Sclerosis Complex: From Basic Science to Clinical Phenotypes. International Child Neurology Association and MacKeith Press, London, UK.

Dickinson M, Selleck M, McMahon A, Bronner-Fraser M (1995) Dorsalization of the neural tube by the non-neural ectoderm. Development 121: 2099–2106.

Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells SA Jr (1993) Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 2: 851–856.

Dottori M, Gross MK, Labosky P, Goulding M (2001) The winged helix transcription factor *Foxd3* suppresses interneuron differentiation and promotes neural crest cell fate. Development 128: 4127–4138.

Edery P, Lyonnet S, Mulligan LM, Pelet A, Dow E, Abel L, Holder S, Nihoul-Fekete C, Ponder BA, Munnich A (1994) Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature 367(6461): 378–380.

Egan CA, Meadows KP, Van Orman CB, Vanderhoof SL (2001) Neurologic variant of epidermal nevus syndrome with a facial lipoma. Int J Dermatol 40: 189–190.

El Shamy WM, Linnarsson S, Lee K-F et al. (1996) Prenatal and postnatal requirements of NT-3 for sympathetic neuroblast survival and innervation of specific targets. Development 122: 491–500.

Flores-Sarnat L (2002) Hemimegalencephaly. Part 1. Genetic, clinical and imaging aspects. J Child Neurol 17: 373–384.

Flores-Sarnat L (2007) Waardenburg syndrome. MedLink (internet medical data system), San Diego.

García-Castro M, Bronner-Fraser M (1999) Induction and differentiation of the neural crest. Curr Opin Cell Biol 11: 695–698.

Gershon MD (1999) Neurotrophins in enteric nervous system development. In: Neurotrophins and the Neural Crest (Sieber-Blum M, ed.), pp. 173–202. CRC Press: Boca Raton, Florida.

Gómez M, Sampson JR, Whittemore VH (eds.) (1999) Tuberous Sclerosis Complex, 3rd ed. New York: Oxford University Press.

Hall BK (1999) The Neural Crest in Development and Evolution. New York Berlin: Springer.

Hall BK (2000) The neural crest as a fourth germ layer and vertebrates as quadroblastic not triploblastic. Evol Devel 2: 3–5.

Hindman BW, Gill HK, Zuppan CW (1997) Primitive neuroectodermal tumor in a child with tuberous sclerosis. Skel Radiol 26: 184–187.

His W (1868) Untersuchungen über die erste Anlage der Wirtbeltierleibes die erste Entwickelung des Hunchens im Ei. Vogel, Leipzig.

Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, Pasini B, Hoppener JW, van Amstel HK, Romeo G et al. (1994) A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 367(6461): 375–376.

Honore SM, Aybar MJ, Mayor R (2003) *Sox10* is required for the early development of the prospective neural crest in Xenopus embryos. Devel Biol 260: 79–96.

Hunt P, Wilkinson DG, Krumlauf R (1991) Patterning of the vertebrate head: murine *Hox-2* genes mark distinct subpopulations of premigratory and migrating neural crest. Development 112: 43–51.

Ikeda I, Ishizaka Y, Tahira T, Suzuki T, Onda M, Sugimura T, Nagao M (1990) Specific expression of the ret proto-oncogene in human neuroblastoma cell lines. Oncogene 5: 1291–1296.

Inoue K, Shilo K, Boerkoel CF et al. (2002) Congenital hypomyelinating neuropathy, central demyelination and Waardenburg-Hirschsprung disease: phenotypes linked by *SOX10* mutation. Ann Neurol 52: 836–842.

Jambart S, Turpin G, de Gennes JL (1979) Neurocristopathies: embryology, physiology, and pathology of the neural crest derivatives. Semaine des Hopitaux 55: 1679–1688.

Jones MC (1990) The neurocristopathies: reinterpretation based upon the mechanism of abnormal morphogenesis. Cleft Palate J 27: 136–140.

Jozwiak S, Janniger CK (2005) Haberland syndrome. eMedicine, 3 May.

Kadonaga JN, Frieden IJU (1991) Neurocutaneous melanosis: definition and review of the literature. J Am Acad Dermatol 24: 747–755.

Kalcheim C (2000) Mechanisms of early neural crest development: from cell specification to migration. Int Rev Cytol 200: 143–196.

Klippel M, Trenaunay P (1900) Du naevus variqueux ostëohypertrophique. Arch Génét Méd (Paris) 3: 611–672.

Krull CE (2001) Segmental organization of neural crest migration. Mech Devel 105: 37–45.

Kuratani S, Matsuo I, Aizawa S (1997) Developmental patterning and evolution of the mammalian viscerocranium: genetic insights into comparative morphology. Devel Dynam 209: 139–155.

Kurokawa D, Kiyonari H, Nakayama R et al. (2004) Regulation of *Otx2* expression and its functions in mouse forebrain and midbrain. Development 131: 3319–3331.

LaBonne C (2002) Vertebrate development: *wnt* signals at the crest. Curr Biol 12: R743–R744.

LaBonne C, Bronner-Fraser M (1998) Neural crest induction in *Xenopus*: evidence for a two-signal model. Development 125: 2403–2414.

LaBonne C, Bronner-Fraser M (2000) *Snail*-related transcriptional repressors are required in *Xenopus* for both the induction of the neural crest and its subsequent migration. Devel Biol 221: 195–205.

Le Douarin N, Kalcheim C (1999) The Neural Crest. 2nd ed. Cambridge University Press, Cambridge, UK.

Liem KF Jr, Tremmi G, Roelink H, Jessell TM (1995) Dorsal differentiation of neural plate cells induced by *BMP*-mediated signals from epidermal ectoderm. Cell 82: 969–979.

Lumsden A, Sprawson N, Graham A (1991) Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo. Development 113: 1281–1291.

McLarren KW, Litsiou A, Streit A (2003) *DLX5* positions the neural crest and preplacode region at the border of the neural plate. Dev Biol 259: 34–47.

Meulemans D, McCauley D, Bronner-Fraser M (2003) *Id* expression in amphioxus and lamprey highlights the role of gene cooption during neural crest evolution. Devel Biol 264: 430–442.

Mokouyama M, Shin D, Britsch S, Taniguichi M, Anderson DJ (2002) Sensory nerves determine the pattern of arterial differentiation and blood vessel branching in the skin. Cell 109: 693–705.

Newgreen DF, Minichiello J (1995) Control of epitheliomesenchymal transformation. I. Events in the onset of neural crest cell migration are separable and inducible by protein kinase inhibitors. Dev Biol 170: 91–101.

Nieto MA, Sargent MG, Wilkinson DG, Cooke J (1994) Control of cell behaviour during vertebrate development by *Slug*, a zinc finger gene. Science 264: 835–839.

Oguma E, Aihara T, Shimanuki Y et al. (1996) Hypomelanosis of Ito associated with neuroblastoma. Pediatr Radiol 26: 273–275.

Ommen GS, McKusick VA (1979) The association of Waardenburg syndrome and Hirschsprung megacolon. Am J Med Genet 3: 217–223.

Pages A (1955) Essai sur le systeme des "cellules claires" de Feyrter. P. Dehan Edt., Montpellier.

Parkes-Weber F (1907) Haemangiectatic hypertrophies of the foot and lower extremity. Med Press, London, UK, pp. 136–261.

Pascual-Castroviejo I (ed.) (2001) Neurofibromatosis. Escuela Libre Editorial, Madrid.

Pascual-Castroviejo I, Roche E, Martínez-Bermejo A et al. (1998) Hypomelanosis of Ito. A study of 76 infantile cases. Brain Dev 20: 36–43.

Pavone L, Curatolo P, Rizzo R et al. (1991) Epidermal nevus syndrome: a neurologic variant with hemimegalencephaly, gyral malformation, mental retardation, seizures and facial hemihypertrophy. Neurology 41: 266–71.

Perris R, Perissinotto D (2000) Role of the extracellular matrix during neural crest cell migration. Mech Devel 95: 3–21.

Puelles L, Rubinstein JL (2003) Forebrain gene expression domains and the evolving prosomeric model. Trends Neurosci 26: 469–476.

Raible DW, Eisen JS (1996) Regulative interactions in zebrafish neural crest. Development 122: 501–507.

Rathjen J, Haines BP, Hudson KM et al. (2002) Directed differentiation of pluripotent cells to neural lineages: homogeneous formation and differentiation of a neurectoderm population. Develoment 129: 2649–2661.

Roach ES, Miller VS (eds.) (2004) Neurocutaneous Disorders. Cambridge University Press, Cambridge, UK.

Rokitansky J (1861) Ein ausgezeichneter Fall von Pigment-Mal mit ausgrebreiteter Pigmentierung der inneren Hirn-und Rückenmarkshäute. Allg Wien Med 1: 113–116.

Romeo G, Ronchetto P, Luo Y, Barone V, Seri M, Ceccherini I, Pasini B, Bocciardi R, Lerone M, Kääriäinen H et al. (1994) Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. Nature 367(6461): 377–378.

Sadaghiani B, Crawford BJ, Vielkind JR (1994) Changes in the distribution of extracellular matrix components during neural crest development in *Xipbophorus* spp. embryos. Can J Zool 72: 1340–1353.

Sánchez-Martín M, Rodríguez-García A, Pérez-Losada J, Sagrera A, Read AP, Sánchez-García I (2002) *SLUG* (*SNAIL2*) deletions in patients with Waardenburg disease. Hum Mol Genet 11: 3231–3236.

Santos CC, Miller VS, Roach ES (2004) Neurocutaneous syndromes. In: Neurology in Clinical Practice (Bradley WG, Daroff RB, Fenichel GM, Jankovic J, eds.) 4th ed., pp. 1867–1900. Philadelphia: Butterworth-Heinemann (Elsevier).

Sarnat HB (2000) Molecular genetic classification of central nervous system malformations. J Child Neurol 15: 675–687.

Sarnat HB, Netsky MG (1981) Evolution of the Nervous System. 2nd ed. Oxford University Press, NY, London.

Sarnat HB, Flores-Sarnat L (2004) Integrative classification of morphology and molecular genetics in central nervous system malformations. Am J Med Genet 126A: 386–392.

Sarnat HB, Flores-Sarnat L (2005) Embryology of the neural crest: its inductive role in the neurocutaneous syndromes. J Child Neurol 20: 637–643.

Selleck MAJ, Scherson TY, Bronner-Fraser M (1993) Origins of neural crest cell diversity. Dev Biol 159: 1–11.

Selleck MAJ, García-Castro M, Artinger KB, Bronner-Fraser M (1995) Dorsalization of the neural tube by the non-neural ectoderm. Development 121: 2099–2106.

Selleck MAJ, García-Castro M, Artinger KB, Bronner-Fraser M (1998) Effects of *Shh* and *noggin* on neural crest formation demonstrate that *BMP* is required in the neural tube but not ectoderm. Development 125: 4919–4930.

Sieber-Blum M (ed.) (1999) Neurotrophins and the Neural Crest CRC Press, Boca Raton, Florida.

Slavin SA, Baker DC, McCarthy JG, Mufarrij A (1983) Congenital infiltrating lipomatosis of the face: clinicopathologic evaluation and treatment. Plast Reconstr Surg 72: 158–164.

Small JM (1955) Some disturbances of the neural crest affecting the nervous system. Proc Roy Soc Med 48: 597–601.

Steijlen PM, Vietor HE, Steensel MV, Happle R (2000) Sweat testing in Hypomelanosis of Ito: divergent results reflecting genetic heterogeneity. Eur J Dermatol 10: 217–219.

Tan SS, Morriss-Kay GM (1985) The development and distribution of the cranial neural crest in the rat embryo. Cell Tiss Res 240: 403–416.

Touraine RL, Attie-Bitach T, Manceau E et al. (2000) Neurological phenotype in Waardenburg syndrome type 4 correlates with novel *SOX10* truncating mutations and expression in developing brain. Am J Hum Genet 66: 1496–1503; *erratum* 2000; 66: 2020.

Trainor P, Krumlauf R (2000) Plasticity in mouse neural crest cells reveals a new patterning role for cranial mesoderm. Nature Cell Biol 2: 96–102.

Unal O, Cirak B, Bekerecioglu M, Kutluhan A, Ugras S, Tali T (2000) Congenital infiltrating lipomatosis of the face with cerebral abnormalities. Eur Radiol 10: 1610–1613.

Van der Hoeve J (1916) Abnorme Länge der Tränenröhrchen mit Ankyloblepharon. Klin Mbl Augenheilk 56: 232–238.

Van der Hoeve J (1920) Eye symptoms in tuberous sclerosis of the brain. Trans Ophthalmol Soc UK 20: 329–334.

Van der Hoeve J (1921) Augengeschwulst bei der tuberosen Hirnskleose (Bourneville). Albrecht Graefes Arch Klin Ophthalmol 105: 880–898.

Van der Hoeve J (1932) Eye symptoms in phakomatoses (The Doyle Memorial Lecture). Tr Ophthalmol Soc UK 52: 380–401.

Villanueva S, Glavic A, Ruiz P, Mayor R (2002) Posteriorization by *FGF*, *Wnt* and retinoic acid is required for neural crest induction. Dev Biol 241: 289–301.

Waardenburg PJ (1948) Dystopia punctorum lachrimarum, blepharophimosis en partiele irisatrophie bij een doofstomme. Ned Tschr Geneeskd 92: 3463–3465.

Waardenburg PJ (1951) A new syndrome combining developmental anomalies of the eyelids, eyebrows and nose root with pigmentary defects of the iris and head hair and with congenital deafness. Am J Hum Genet 3: 195–253.

Woda JM, Pastagia J, Mercola M, Artinger KB (2003) *Dlx* proteins position the neural plate border and etermine adjacent cell fates. Development 130: 331–342.

Yakovlev PO, Guthrie RH (1931) Congenital ectodermoses (neurocutaneous syndromes) in epileptic patients. Arch Neurol Psychiat 26: 1145.

Zhang W, Simos PG, Isibashi H (2003) Neuroimaging features of epidermal nevus syndrome. Am J Neuroradiol 24: 1468–1470.