

## Interactions Between the Middle and Inner Ear in Patients with Ear Malformations

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We sought to further evaluate interactions between the middle and inner ear during embryogenesis resulting in ear malformations, and undertook a clinical investigation of such malformations. Four ear malformation syndromes with different aetiologies and an animal model of known aetiology were compared. By comparing patients with thalidomide induced ear malformations, where the exact time of administration of the teratogen was known, the sensitive time for ear development was obtained. The severity of the ear malformation was related to the time period during which the ear formed. Thus, patients with mandibulofacial dysostosis and hemifacial microsomia with severe middle ear malformations, were damaged during embryonic days 21–24. Patients with CHARGE association with minor middle ear malformations but severe inner ear malformations were damaged during embryonic days 24–29. Therefore, malformations of the middle and inner ear occur during a short time period of embryogenesis. A plausible explanation for the different malformations is related to disturbed or reduced neural crest cell migration during embryogenesis. *Key words: ear malformations, syndromes, genetic, teratogen, neural crest.*

### INTRODUCTION

Ear malformations are estimated to occur in about 150 per 100 000 newborns. The most common malformations affect the external ear, ear canal and middle ear. Malformations of the inner ear are less common. Almost 50% of ear malformations occur spontaneously without known cause. The rest occur in combination with a syndrome of which several are hereditary. Furthermore, some ear malformations occur as a result of teratogenic exposure. Generally, it is believed that the different routes by which the inner and middle ear forms during the embryonic period, generate differences in the incidence of malformations. The underlying mechanisms of how different ear malformations occur, is however, unknown. The present investigation was undertaken to gain more knowledge of interactions between the middle and inner ear during embryonic life resulting in ear malformations.

### MATERIALS AND METHODS

Patients evaluated and treated for ear malformations at the ENT clinic, Sahlgrenska University Hospital, between 1988 and 2002 were investigated. Investigations comprised clinical evaluation, X-ray investigations with computed tomography (CT) or magnetic resonance tomography (MRT). Hearing was evaluated by pure tone audiometry and bone conduction audiometry. In infants, brain stem audiometry was used to evaluate hearing at ages when standard audiometry was not possible to perform. Patients with vestibular malformations were

tested with electronystagmography (ENG) or a comparable rotational test.

Patients with four different syndromes were especially studied. These were mandibulofacial dysostosis (MFD, Treacher Collins syndrome), hemifacial microsomia (HFM, Goldenhar syndrome), CHARGE associations and thalidomide induced embryopathy (TE).

An animal model was developed to induce ear malformations comparable with the clinical counterparts (1). Pregnant Sprague-Dawley rats were given etretinate (Tigason<sup>®</sup>, Roche Co, Skärholmen, Sweden) at embryonic day 9.5 at a dose of 10 mg/kg. Embryos were collected during embryonic days 13–20 and processed for routine histology after serial sectioning and staining (2).

### RESULTS

Three hundred and forty patients were evaluated. Of these, 192 were male and 148 were female. The malformation was bilateral in 135 cases. The right ear was affected in 92 monaural cases and the left ear in 78 monaural cases, and thus 440 ears were malformed. Isolated microtia occurred in 184 cases, and in the other 156 the ear malformation occurred as part of a syndrome. Of the syndromes, HFM was the most common, occurring in 52 cases, CHARGE in 34, MFD in 29 and TE in seven cases. Typical findings of the ear from a patient with CHARGE association are shown in Figure 1.

In Table 1 the variations of external ear malformation in relation to syndrome are shown. As can be seen, severe malformations were common in MFD, HFM and TE but



(a)



(b)

Fig. 1. a) Eight-year-old boy with CHARGE association. The external ear is slightly malformed with a 'web' or 'snip-off' defect. b) A CT scan from the inner ear of the same patient shows that the semicircular canals are missing.

not in the CHARGE association. Table 2 shows the variations of middle ear malformations in relation to syndrome. As can be seen, severe malformations were common in MFD, HFM and TE but not in the CHARGE association. In Table 3 inner ear malformations are presented. Inner ear malformations were most common in CHARGE association and in the TE syndromes.

In the animal model it was possible to induce a spectrum of malformations of the external ear, which to a high extent resembled the clinical counterparts (2). The embryonic rat ear is sensitive to teratogens during a very

Table 1. Malformations of the external ear according to Meurman (1958) Meu 3 – severe; Meu 2 – average; and Meu 1 – slight malformation. MFD – mandibulofacial dysostosis; HFM – hemifacial microsomia; CHA – CHARGE association; TE – thalidomide embryopathy.

Per cent	MFD	HFM	CHA	TE
Meu 3	52	56	0	69
Meu 2	19	25	8	31
Meu 1	29	19	92	0

Table 2. Malformations of the middle ear according to Altmann (1958) and Creemers (1984). AC 3 – severe; AC 2B – less severe; AC 2A – average and AC 1 – slight malformation. MFD – mandibulofacial dysostosis; HFM – hemifacial microsomia; CHA – CHARGE association; TE – thalidomide embryopathy.

Per cent	MFD	HFM	CHA	TE
AC 3	36	9	0	50
AC 2B	45	63	0	50
AC 2A	8	31	16	0
AC 1	11	0	84	0

Table 3. Malformations of the different parts of inner ear in relation to syndrome MFD – mandibulofacial dysostosis; HFM – hemifacial microsomia; CHA – CHARGE association; TE – thalidomide embryopathy.

Per cent	MFD	HFM	CHA	TE
Cochlea	2	4	33	42
Vestibule	0	0	15	15
Canals	0	0	45	15

short time period (<24 hours). The most prominent feature of teratogenic exposure was a reduced migration of neural crest cells, which occurred during embryonic day 9–10 (3).

When correlating to the known time for intake of thalidomide, creating a syndrome with ear malformations in humans, the sensitive period for the external and middle ear would be between embryonic day 21 and 24, and for the inner ear between embryonic day 24 and 29 (4, 5).

## DISCUSSION

The constituents of the ear are known to be derived from different embryonic origins. Using the quail-chick chimera technique, it has been shown that the different parts of the ear and temporal bone are formed from neural

Table 4. *Paralysis of the abducens and facial nerves in relation to syndrome MFD – mandibulofacial dysostosis; HFM – hemifacial microsomia; CHA – CHARGE association; TE – thalidomide embryopathy.*

Per cent	MFD	HFM	CHA	TE
Abducens	0	30	33	86
Facial	0	15	15	43

crest, cephalic mesoderm, paraxial mesoderm and first somite mesoderm (6).

Ear malformations are probably hereditary in a high proportion of cases, but the chromosomal defects are presently not fully established. Of known chromosomal defects, 5q31-q33 deletion, 4p14-p15 deletion and 6p21 deletions have been described for MFD (7–9). HFM has been shown to be related to trisomy 22 and 22q11 deletion (10). A candidate gene, the Goosecoid gene, has been suggested for HFM (11). CHARGE association has been related to trisomy 18, trisomy 19q and monosomy 21q, t/2, 7Xp14, q21.11 translocation (12). Candidate genes for the MFD and HFM syndromes can be drawn from known proteins such as extracellular matrix proteins (ECM), neural cell adhesion molecules (NCAM) or fibronectin. These are all known to work in relation to migration of the neural crest during the critical period for external and middle ear malformations. A candidate gene for the CHARGE association could be the PAX2 gene (13). This is known to function in all tissues affected by malformations, including the inner ear. Other genes could act during the sensitive time periods by 'on' or 'off' action such as structural proteins or functional proteins (enzymes), the exact mechanism being presently unknown.

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