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Review Article

The Genetic Basis and the Mode of Inheritance of the Outer Ear Development; A review

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Abstract

The outer ear shape is of high importance to all species. Several anomalies can be seen that cause reduction or even elongation in it, here and through this review i was trying to summaries the molecular basis and the mode of inheritance for some of these anomalies including microtia and anotia in animals and humans with special references to that occurs in sheep.

Key words: Microtia; anotia; genetics; Ear development

Introduction

The ear is an auditory organ which collects outside sounds waves and converts it into nerve impulses sent to central nervous system, and then the brain interprets these impulses into a voice, the visible auricle developed to capture and funnel sound waves more effectively. Besides sound perception, the ear also holds different functions, like heat dissipation and body balance (Webster) [1-5].

Microtia, the hereditary under development of the outer ear, occurs in many species including humans, mice and various livestock species, while Anotia that is the total absence of auricle. Microtia and Anotia exists both as an isolated trait or as part of various syndromes. In sheep, microtia generally is an isolated condition. The Awassi, a fat-tailed sheep from southwest Asia, is among the breeds showing microtia, being of the isolated type, microtia in the Awassi is not associated with other defects (Jawasreh), However, it reduces the market value of the defected individuals because of its undesirable characteristic to breeders (Jawasreh). In humans, the anotia or microtia usually associated with some abnormalities in the face and jaw, kidney and heart problems, and vertebral deformities (Luquetti) [6-10].

In domestic animals, there are few investigations focused on the non-economic characteristics such as ear size. Also, there are large differences in research's results in terms of the genetic locus of the mutation(s) that cause microtia in different sheep breeds.

Jawasreh were the first who studied the genetic basis of microtia in sheep using GWAS (Genome-wide association study) and they suggested GATA6 (GATA binding protein 6) as a candidate gene for this mutation in Awassi sheep. Thereafter, Gao conducted a GWAS and reported DCC (deleted in colorectal carcinoma) as a candidate gene responsible for the variations in ear size observed in Duolang sheep. Also, Mastrangelo suggested CLRN1 (Clarin 1) as a causative gene of microtia in Valle del Belice sheep. Recently, used GWAS and DNA (Deoxyribonucleic acid) fragments sequencing for detecting the genetic cause of microtia in Altay sheep and suggested a duplication within an evolutionary conserved region (ECR) near HMX1 (H6 family home-box 1) gene as a causative mutation of microtia. The objective of this review is to gather all efforts found worldwide that explained the mode of inheritance and the genes that were suggested to affect the outer ear development in Human and farm animals [11-20].

Microtia Definition, Types, Occurrence and General Information's

Outer ear is composed primarily of the pinna. Pinna is elastic cartilage covered by skin, in microtia case there is no cartilage in the lobe rather fibro-fatty tissue (Kalam).According to Alasti Microtia is defined as a hereditary congenital deformity of the outer ear, where the external ear is underdeveloped with phenotypes varying from a small auricle to total absence of pinna, while Carey consider Anotia as severe case of microtia

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and defined it as a total absence of the ear and external auditory canal. There are four types of microtia. One of them is the mildest form, where the ear retains its normal shape, but is smaller than usual, and the most severe case where the whole external ears structures are disappeared (Anotia). This condition can affect one or both ears (Canfield)

Microtia occurs in several species including sheep (Lush, 1930 and Dennis), goats (Basrur), dogs (Messas), human (Hunter and Yotsuyanagi), mice (Luquetti), Rabbits (Tanchev) and swine (Ren), but, Elias and Benettpointed out that the microtiais more prevalentin sheep in comparison to theother ruminants and it is one of the most common congenital abnormalities in sheep. Soundararajan conducted a study to determine the occurrence of microtia in Madras Red sheep and reported 6.62% overall occurrence of microtia which 50% in adult females, 21.53% in young males, 15.38% in young females, 7.69% in female lambs, 3.85% in adultmales and 1.92% in male lambs.Few researches recently described microtia in sheep, Jawasreh Consideredmicrotia in Awassi sheep breed as an isolated type and it is not associated withother defects and appears to be simply inherited; ears have three distinct phenotypes: normal, earless and small. while the ear type of Duolang sheep has been discussed by Goa) who divided it to large or floppy ears with a large variation in ear size. Mastrangelo described ear type in Valle del Belice sheep, dairy breed from southern Italy, and they divided it in only two distinct phenotypes (normal and small).

Several researchers linked microtia to hearing loss in human, up to 80% of patients with microtia have aural atresia and suffering from conductive hearing loss, with air conduction hearing reduced by 40–65 decibel while bone conduction is normal in most of the affected individuals ears (Stewart and Downs.Suutarla and Alasti), However, Pere studied some morphological traits (Body weight, Thoracic girth, Cannon perimeter, Ear length, Head length and Face length) and their association with microtiain Guatemala native sheep, they did not found any differences in morphology between microtic and non-microtic sheep. But, according to Mastrangelo who did several interviews with Valle del Belice sheep breeders, microticsheep has higher milk production compared to that normal ear phenotype. Jawasrehet reported that the tendency of the earless sheep to be more nervous and they explained it by the hearing weakness of those microtic sheep. In addition to,Alasti and Van Camp, (2009) linked ear to other physiological functions and environmental adaptation. Blaxter studied the association between heat dissipation and body surface area beside feeding level and physiological state and reported that the bodies of higher surface area are exposure to lose more heat than those of smaller area. Depending on that, Goa reported that the sheep with microtia have less surface area and exposure to lose less heat that those have normal ear shape [21-30].

Genetic Basis of the External Ear Shape Inheritance

Human

In human, many research workers focused on congenital anomalies found in Human. Hunter and Yotsuyanagi (2005) divided ear congenital anomalies into three major categories that are: Grade 1: dysplasia, where microtia (shortness or absence of pinna), lobular anomalies and tragal anomalies, in this case patients have irregular ear shape but usually no extra cartilage or skin required when surgical ear re-construction is requested; Grade 2: dysplasia: cup and mini ear. The outer ear lacks a part of the normal ear and the reconstruction surgery requires cartilage and skin substitution. Grade 3: dysplasia: bilateral or unilateral Anotia and Microtia, patients in this case required total ear reconstruction surgery. According to Luquetti the occurrence of microtia was ranged between 0.83 to 17.4 per ten thousand births in France, Sweden, Italy, United States of America and Finland. Also, there are many genes were involved as causative genes that associated with microtia in human, among those genes FRAS1(Fraser extracellular matrix complex subunit 1), FREM2 (FRAS1 related extracellular matrix 2), MLL2 (lysine methyl transferase 2D), GDF6 (growth differentiation factor 6), FGFR2 (ibroblast growth factor receptor 2), FGFR3 (ibroblast growth factor receptor 3), FGF10 (fibroblast growth factor 10), HOXD (HOXD antisense growth-associated long non-coding RNA), ORC1 (origin recognition complex subunit 1), ORC4 (origin recognition complex subunit 4), ORC6 (origin recognition complex subunit 6), CDT1 (chromatin licensing and DNA replication factor 1), CDC6 (cell division cycle 6), HOXA2 (homeobox A2), DHODH (dihydroorotate dehydrogenase (quinone)), HMX1 ,GLI3 (GLI family zinc finger 3), SALL1 (spalt like transcription factor 1) and TCOF1 (treacle ribosome biogenesis factor 1). Like other birth defects Duan, (2013) claimed microtia to be as a result of genetic or environmental predisposition, such as the exposure to retinoic acid, mycophenolate mofetil and thalidomide during pregnancy can cause microtia [31-35].

Animal Models for the External Ear Shape

Jawasrah suggested sheep as an important model for understanding the genetic basis of microtia in human. Ear growth and development need several steps to be completed: cranial neural crest patterning, first and second branchial arches' outgrowth, chondrocytes progenitor condensation, and cartilage formation, all proteins and genes associated with ear growth and development take part in these steps. On the other hand, King found a relationship between BMP5 (Bone morphogenic 15) gene and external ear development in mice. Also, Fgfr 1 (Fibroblastgrowth factor receptor 1) associated with ear shortness in the same species. In the same context, mutations in Hoxa1, Hoxa6, Hoxb7 and Hoxb1 (Homeobox A cluster 1,6,7 and Homeobox B cluster 1) in mice can lead to ear malformations (Santagati) [36-40].

Boyko studied ear shape and divided it into three categories (prick, inter mediate and floppy) in dogs and found a single region (CFA10: 10-11.6 Mb) beside MSRB3 (Methionine sulfoxide reductase B3) gene which related to this trait using 915 dogs from 80 breeds [41-45].

Domestic Animals

In ruminants, Madhavan and Ajithkumar, reported that a rare hereditary case of microtia in goats that associated with deafness and lacking of external ear canal on the left size ear. According to Basrur and Yadav, Microtia and Anotiaareof the most abundant congenital malformations in ovine and have a genetic origin, they also considered ear length as incomplete dominant trait. The Anotia sheep suffer from deafness, but no differences were detected in inner ear (Hamori) [46-50].

Yamane, (1915) was the first researcher who reported ear crop in the cattle. After 7 years, lush, (1922) reported ear deformity that dominantly inherited and was not associated with syndrome in Jersey cattle, the author was noticing a notch at the lower edge of the cattle ears. According to Koch cattle with notched, crop or variably niched ears in some cases associated with ear cartilage deformation and pinna shortness and this occurs in both genders, but the cattle breeders did not notice any alteration in hearing ability. Also, they performed a GWAS, whole genome sequence and Sanger sequence for 36 normal and 32 affected Highland cattle and suggest HMX1 as a causative gene responsible for ear crop in bovine [51-55].

Artificial selection increases the rapid phenotypic evolution in swine, a different morphology of ears seems to be associated with multiple genes. Guo found a strong association between SSC6 (Susscrofa chromosomes) gene and ear shape in pigs, but Li suggested *HMGA2* on chromosome 5 *as a strongest* causative gene for differences in ear size in pigs. Other studies suggested other genes to be related to microtia in pigs: GDF11 (Wei), SOX5 (SRY (sex determining region Y)-box 5) and PTHLH (Parathyroid hormone-like hormone), (Vaysse) [56-60].

Sheep

Recently, just few researches were studied the genetic basis of microtia in sheep. Jawasreh found a single nucleotide polymorphism (SNP) at locus 34647499 on chromosome 23 that statistically associated with microtia in Awassi sheep using a genome wide association study (GWAS). This SNP located in intergenic region between GATA6 and MIB1 (mindbomb E3 ubiquitin protein ligase 1) gene and adjacent to the encoding transfer RNA of Arginine as illustrated in (Figure 1). They suggested GATA6 as the most candidate gene that responsible to Microtiain this sheep breed, because its function and it is closer to the discovered SNP than transfer RNA gene [61].

Gao found 38 SNPs at chromosome level were significantly associated with ear shortness in Duolang sheep breed, but just one SNP (rs462740419) was significantly related to this trait at Genome-wide level. This SNP located within DCC gene (Deleted in Colorectal Carcinoma) in intron 2. They also suggested two additional genes to be related to ear size in sheep: SOX5 (SRY-box5) and PTPRD (Protein tyrosine phosphatase receptor type D) [62].

Mastrangelo collected a blood samples from 40 sheep individuals, 20 normal ear sheep and 20 with microtia, and performed a GWAS to identify the genetic locus for mutations that probably associated with microtia in Valle del Belice sheep breed. They found a single SNP, located at 23515286 bp on chromosome 1 (rs419889303). This SNP (Guanine to Adenine) within CLRN1 (Clarin 1) gene intron 3 that was significantly associated with microtia, the authors suggested a CLRN1 as a candidate gene for microtia in sheep [63].

He did a Genome-Wide association study (GWAS) to find the locus of causative mutation(s) for dominantly inherited microtia in Altay sheep, they collected blood samples from 29 normal animals and 26 with microtia, and found a strong SNP (P = 2.99 E ^-10) positioning on 114154235 bp within ovine chromosome 6, this locus is in evolutionarily conserved region (ECR) beside H6 family home box 1 (HMX1) gene. After GWAS, they amplified part of ECR near HMX1 gene from 25 normal, 49 with microtia and 123 samples from other sheep breed using sanger PCR and tested product size using agarose gel; the bands have taken the following patterns: relatively large band in mutant samples (+76 bp), two bands (large and small) in heterozygous and relatively small in normal samples (same as wide type). Finally, they suggested a duplication of 76bp in ECR surrounding HMX1 gene as a causative mutation of microtia in sheep [64].

Recently, Jawasreh and Alomari investigated the mode of inheritance of the outer ear concentrating on the Microtia, and they observe mutations in only two genes significantly associated with Microtia in Awassi: duplication in ECR near HMX1 and SNPs at GATA6 exon 7. Sequence alignment revealed that the ECR locus accounts for the microtia phenotype, while GATA6 exon 7 acts as a modifier gene [65].

GATA6 Gene Functions and its Role in Chondrogenesis and Ear Development

GATA6, a gene, is a member of a zinc finger translation factors group that contributes incell differentiation and organogenesis during vertebrate development process. GATA family plays an important role in reproductive cells development that acts as mediators to reproductive function. According to Lowry GATA family divided into two sub-families depends on its function, structure and locus. GATA-1, 2 and 3 factors are often associated with the nervous system development and hematopoietic cell lineages (Cantor), while Molkentin reported that GATA4,5and6 members are usually commitment with organ development, including the gut, heart, vasculature. During early embryogenesis GATA6 gene is expressed and confines to endo-and mesodermal derivedcells during later embryogenesis and along these lines assumes a significant role in gut, lung, and heart advancement, changes in this gene are claimed to be related with many congenital defects. Brewer) found new GATA6 expression within locus of chondrogenesis derived from cranial neural crest and sclerotomes. Also, According to Alexandrovich reported that GATA6 assumes a job in chondrogenesis and Gpr49 (G protein-coupled receptor 49) is a potential direct target of GATA regulation procedure. Narita stated that GATA6 are expressed in mouse smooth muscle. While the loss of expression of GATA 6 and GATA 4 that expressed in the ovary lead to down-regulation of genes regulated the ovarian developmental process (Fst (follistatin) and Irx3 (iroquoishomeobox 3)) and decrease expression of the

granulosa and pre-granulosa cell markers FOXL2 (forkhead box L2) and SPRR2(Small proline-rich protein 2), respectively (Padua) [66].

DCC Gene Functions and its Role in Chondrogenesis and Ear Development

DCC gene is a member of the immunoglobulin super family of cell adhesion molecules and work as a transmembrane dependence receptor for netrins, and plays a critical role in the regulation of axon guidance during differentiation of the central nerve system. According to Matilainen DCC affect ear development by co-functioning with the netrin 1 gene, which involved in inner ear development. Keino reported that the DCC gene to encodes for a netrin receptor, which is associated with the alteration from proliferation to terminal development in different tissues, which goes along with Yagami, finding that Netrin-1 was highly expressed in immature chondrocytes of cartilage-like tissues. Also, Bosserhoff linked between DCC gene signaling and migration of CD166 (chondrogenic progenitor cells) to sites of cartilage damage in human.

CLRN1 Gene Functions and its Role in Chondrogenesis and Ear Development

CLRN1 (clarin1) is a four-pass membrane domain protein with homology with the tetras panin family (Adato), this family of proteins that contain connexins and Claudins that cause when mutated hearing loss in humans (Duman and Tekin). According to Phillips CLRN1 cause Usher syndrome type 3A (an autosomal recessive disorder characterized by gradual hearing and vision loss). Geller was found CLRN1 gene expression in multiple areas of the body, including hair cells (sensory cells in the inner ear that facilitate sound and motion signals transition to the central nervous system). Also, Zallocchi reported that CLRN1 plays a possible role in communication between eye retina and nerve cells in the inner ear [67].

HMX1Gene Functions and its Role in Chondrogenesis and Ear Development

In embryos of mammals and birds, H6family home-box 1 (HMX1) gene is expressed in peripheral ganglia, branchial arches and eyes (Yoshiura; Wang). Also, in human and mice, Munroe reported a mutation on HMX1 coding region that causea recessive phenotype. Moreover, a deletion of 5777 bp including around 600 bp from evolutionarily conserved region located 79 kp from HMX1 gene is associated with suppression of craniofacial mesenchyme HMX1 expression (Quina). Also, according to Schorderet mutation on HMX1 gene cause malformation in the Human external ear. Rosin reported that a cooperative work between ECR in Hoxa2 gene and Pbx and Meis transcription factors regulate expression of HMX1.In addition to that, HMX1 mutation affecting both: middle and external ears (Anthwal and Thompson).

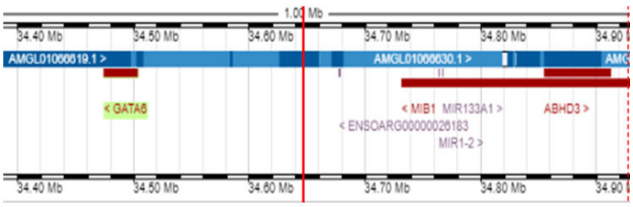

Figure 1: The genomic locus of SNP 34647499 on chromosome 23, the vertical red line represents the exact locus of this SNP.

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