Three candidate genes for hearing loss

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- We analyzed a group of congenital hearing loss patients with the goal of identifying possible causative genes. The patients had a wide spectrum of clinical presentations; the genetic underpinnings are little understood.
- Hearing loss is one of the most common genetic disorders. It is estimated that as many as 70% of congenital hearing loss cases have a genetic cause. Of these, about 30% have syndromic hearing loss. Over half of non-syndromic hearing loss cases have no known genetic cause. Many genes have been implicated in hearing loss, making it a highly heterogeneous disorder. Our focus is on non-syndromic hearing loss, which typically features small deletions or duplications within a gene or regulatory element.
- SNP (single nucleotide polymorphism) arrays are used to identify deletions and duplications, or copy number variations (CNVs). We used Illumina 550K SNP genotyping arrays to find CNVs associated with non-syndromic forms of hearing loss. This array differs from older methods in that it gives us a high-resolution picture of the genome, allowing us to see small changes.
- Not all CNVs are pathogenic –unaffected individuals have 30 of these CNVs on average. The prevalence of benign CNVs makes identifying pathogenic ones difficult. A program called Perl Copy Numbers of Potential Interest or PECONPI ranks these CNVs based on how likely they are to be pathogenic. The samples used in this step came from patients with enlarged vestibular aqueducts, which is associated with Pendred Syndrome. The genetic basis of this syndrome is not well understood. Patients with and without EVAs were used for sequencing.

We focused on those genes that received a score of 25 or higher in PECONPI and selected 3 of those genes: **PCDH20**, **SLC2A4RG** and **CRISPLD2**.

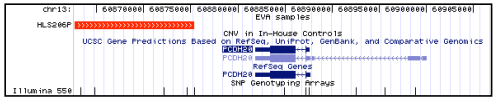
	Call No.	Sample ID	Simple Chr	Simple State	Sample Size	Simple #ofSNPs	Bands	Literature Ovlp%	InHouse#	Total Cvg%	Corrected Cvg%	Perfect Matches	Simple Ovlp#	Genes#	Unique Genes#	Unique Genes (Primorric	Score	Hyperlink
2	3101	HLS253P	Chr7	het	514,994	105	7q35		1	55%				1	1	*CNTNAP2	35.5	link
3	3728	HLS150P	Chr8	hom	3,743	5	8q13.3		7	31%			4	1	1	*EYA1	33.1	link
4	2979	HLS051P	Chr7	het	148,736	27	7p22.1							4	4	FLJ10324; KIAA0415; MMD2; PAPOLB	29.2	link
5	4118	HLS058P	Chr10	het	93,497	27	10p12.1							1	1	PTCHD3	28.6	link
6	3650	HLS072P	Chr8	het	62,025	24	8p21.3							2	2	ChGn; SH2D4A	28.0	link
7	5215	HLS206P	Chr13	het	25,348	13	13q21.31							1	1	PCDH20	26.6	link
8	3580	HLS138P	Chr8	het	9,702	13	8p23.1							1	1	DEFB1	26.4	link
9	6624	HLS251P	Chr20	het	34,421	9	20q13.33	19%						4	4	LIME1; SLC2A4RG; ZBTB46; ZGPAT	26.2	link
10	6673	HLS094P	Chr20	het	23,352	7	20q13.12							1	1	SPINT4	25.9	link
11	6852	HLS053P	Chr21	het	33,159	6	21q22.3							1	1	C21orf70	25.9	link
12	6011	HLS046P	Chr16	het	28,365	21	16q24.1		1	50%				2	2	ATP2C2; KIAA1609	25.9	link
13	6014	HLS051P	Chr16	het	18,449	7	16q24.1							1	1	CRISPLD2	25.9	link
14	15	HLS177P	Chr1	het	12,260	6	1p36.13							3	3	CLCNKA; CLCNKB; FAM131C	25.7	link
15	4963	HLS160P	Chr12	het	2,386	6	12q13.2							1	1	OR9K2	25.6	link
16	4276	HLS033P	Chr10	het	11,895	11	10q21.1		1	55%				1	1	MBL2	24.6	link
17	889	HLS033P	Chr3	het	207,994	111	3p14.2							1	1	(FHIT)	24.1	link
18	226	HLS111P	Chr1	het	3,932	3	1p13.3		1	100%		1		1	1	C1orf62	22.3	link
19	3896	HLS072P	Chr9	het	166,424	40	9p21.1	100%	1	100%		1	1	1	1	LINGO2	20.9	link

1. Collect samples from probands, prepare DNA
2. DNA analyzed by SNP array probe, results visualized in BeadStudio
3. Array data processed by CNV-calling algorithm (PennCNV) and intervals of CNV listed
4. Intervals of CNV are analyzed against a control set; scored and ranked; displayed in Excel (see figure above).
5. Sequence candidate genes

The Candidate Genes:

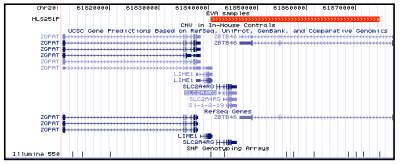
PCDH20: protocadherin 20 precursor (13q21.31)

- Undetermined function, but is thought to be involved in calcium-dependent cell adhesions in the brain
- PCDH15 linked to Usher syndrome type 1F



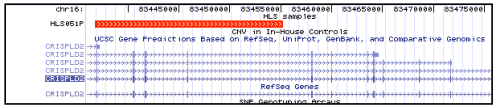
SLC2A4RG: solute carrier family 2 member 4 gene regulator (20q13.33)

- Activates transcription of SLC2A4 AKA GLUT4
- SLC26A4 is implicated in Pendred syndrome
- Insulin-regulated facilitated glucose transporter



CRISPLD2: cysteine-rich secretory protein LCCl domain-containing 2 precursor (16q24.1)

- Candidate for nonsyndromic cleft palate



Results & Future Directions

- At least 2 potentially harmful changes were found in the exons we sequenced according to PolyPhen
- The changes found in PCDH20 and SLC2A4RG were found in EVA patients and in other patients
- Not all exons were sequenced and not all sequences were clean – there are likely more changes in these genes which were not uncovered
- We want to focus on regulatory elements outside of coding regions next. Changes in regulatory elements do not affect gene expression throughout the entire body as changes in the coding regions do. This would allow us to identify mutations involved in cases of isolated hearing loss.

Changes found in each gene

PCDH20		
117C>A, S39R	12 het, 1 hom	possibly damaging
732A>G, V244V	1 het	no change
1314G>T, V438V	>50%	no change
1481C>T, P494L	1 het	benign
1600A>G, I534V	3 het	benign
2082G>A, L694L	1 het	no change
SLC2A4RG		
365G>A, G122E	3 het	probably damaging
372G>A, P124P	>50%	no change
381C>T, A127A	1 het	no change
CRISPLD2		
12C>G, V4V	1 het	no change
21T>A, G7G	1 het	no change
313A>G, S105G	11 het	polymorphic
581T>A, V194V	1 het	no change
965C>G, T322S	25 het	polymorphic