## **Table 1 Patient characteristics and results of univariate analysis using CTCAE & Chang grading as ordinal outcomes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | CTCAE | P value\*\* | Chang | P value\*\* |
|  | **0** **(n =26)** | **1** **(n=8)** | **2****(n=41)** | **3****(n=35)** | **4****(n=6)** |  | **0****(n=31)** | **1a****(n=17)** | **1b****(n=15)** | **2a****(n=5)** | **2b****(n=10)** | **3****(n=32)** | **4****(n=5)** |  |
| Age (years) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Median  | 7.73 | 12.78 | 8.80 | 4.94 | 3.95 | 0.010 | 8.49 | 9.66 | 8.80 | 4.41 | 6.43 | 5.24 | 4.04 | 0.040 |
| Range | 0.59-17.67 | 0.83- 18.60 | 0.80, 18.18 | 0.62, 17.15 | 1.17, 10.05 |  | 0.69, 18.03 | 1.98, 18.18 | 0.80, 18.60 | 1.22, 14.02 | 1.20, 17.15 | 0.62, 15.98 | 1.17, 10.05 |  |
| Ethnicity |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Caucasian | 23(22.8%) | 7 (6.9%) | 33(32.7%) | 33(32.7%) | 5 (5.0%) | 0.45 | 27(27.0%) | 13(13.0%) | 12(12.0%) | 4 (4.0%) | 9 (9.0%) | 31(31.0%) | 4 (3.0%) | 0.62 |
| Other | 3 (25.0%) | 0 (0.0%) | 6 (50.0%) | 2 (16.7%) | 1 (8.3%) |  | 3 (25%) | 3 (25%) | 2 (16.7%) | 1 (8.3%) | 1 (8.3%) | 1 (8.3 %) | 1 (8.3%) |  |
| Gender |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Male | 17 (23.0%) | 6 (8.1%) | 32(43.2%) | 15(20.3%) | 4 (5.4%) | 0.030 | 22(30.1%) | 10(13.7%) | 13(17.8%) | 4 (5.5%) | 7 (9.6%) | 14(19.2%) | 3 (4.1%) | 0.089 |
| Female | 9 (21.4%) | 2 (4.8%) | 9 (21.4%) | 20(47.6%) | 2 (4.8%) |  | 9 (21.4%) | 7 (16.7%) | 2 (4.8%) | 1 (2.4%) | 3 (7.1%) | 18(42.9%) | 2 (4.8%) |  |
| Cumulative cisplatin dose in mg/m2 |  |  |  |  |  |  |  |  |  |  |  |
| Median | 344 | 317.0 | 480 | 320 | 260 | 0.023 | 350 | 480 | 480 | 400 | 440 | 280 | 280 | 0.012 |
| Range | 60-600 | 240-560 | 208-560 | 180-560 | 100-800 |  | 60-600 | 208-560 | 240-560 | 280-480 | 216-516 | 180-560 | 100-800 |  |
| Cranial Irradiation |  |  |  |  |  |  |  |  |  |  |  |  |  |
| YES | 3 (7.5%) | 2 (5.0%) | 16(40.0%) | 17(42.5%) | 2 (5.0%) | 0.028 | 4 (10.0%) | 5 (12.5%) | 6 (15.0%) | 3 (7.5%) | 4(10.0%) | 16(40.0%) | 2 (5.0%) | 0.048 |
| NO | 23 (30.3%) | 6 (7.9%) | 25(32.9%) | 18(23.7%) | 24(5.3%) |  | 27(35.5%) | 12(15.8%) | 9 (11.8%) | 2 (2.6%) | 6 (7.9%) | 16(21.0%) | 3 (4.0%) |  |
| Vincristine  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| YES | 9 (14.3%) | 2(3.17%) | 22(34.9%) | 25(39.7%) | 5(7.94%) | 0.0091 | 10(15.9%) | 8 (12.7%) | 7 (11.1%) | 3 (4.8%) | 5 (7.9%) | 25(39.7%) | 4 (6.4%) | **0.012** |
| NO | 17 (32.1%) | 6(11.3%) | 19(35.8%) | 10(18.9%) | 1(1.89%) |  | 21(39.6%) | 9 (17.0%) | 8 (15.1%) | 2 (3.8%) | 5 (9.4%) | 7(13.2%) | 1 (1.9%) |  |
| Carboplatin\* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| YES | 2 (9.52%) | 0 (0%) | 8 (38.1%) | 10(47.6%) | 1(4.76%) | 0.097 | 2 (9.52%) | 2 (9.52%) | 3 (14.3%) | 0 (0%) | 1(4.76%) | 11(52.4%) | 1(4.76%) | 0.076 |
| NO | 24 (25.3%) | 8(8.42%) | 33(34.7%) | 25(26.3%) | 5(5.26%) |  | 29(30.5%) | 15(15.8%) | 12(12.6%) | 5 (5.26%) | 9(9.47%) | 21(22.1%) | 4(4.21%) |  |

Data are presented as number (%) of patients, or median with range (minimum, maximum), unless otherwise indicated. Analysis is of the worse ear if asymmetric hearing loss present. Cumulative cisplatin dose is measured in mg/m2. \*Carboplatin YES refers to patients who cisplatin and carboplatin as part of the same treatment protocol. NO refers to patients who were not exposed to carboplatin whilst they were also treated with cisplatin. Patients in the latter group may have been changed from cisplatin to carboplatin therapy during their treatment.\*\*p-value from multinomial logistic regression model.

## **Table 2: Summary statistics for genetic data CTCAE grading for *COMT*, *TPMT* and *ACYP2*** **CTCAE criteria using worse ear in cases of asymmetric hearing loss**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *COMT* |  | Grade 0(n=26) | Grade 1(n=8) | Grade 2(n=41) | Grade 3(n=35) | Grade 4(n=6) |
| rs9332377 | CC | 18 (22.5%) | 4 (5%) | 28 (35%) | 27 (33.8%) | 3 (3.8%) |
| CT | 7 (21.1%) | 3 (9.4%) | 12 (37.5%) | 7 (21.9%) | 3 (9.4%) |
| TT | 1 (25%) | 1 (25%) | 1 (25%) | 1 (25%) | 0 (0%) |
| rs4646316 | CC | 18 (26.1%) | 4 (5.8%) | 24 (34.8%) | 19 (27.5%) | 4 (5.8%) |
| CT | 6 (14.3%) | 4 (9.5%) | 14 (33.3%) | 16 (38.1%) | 2 (4.8%) |
| TT | 1 (33.3%) | 0 (0%) | 2 (66.7%) | 0 (0%) | 0 (0%) |
| *TPMT* |
| rs12201199 | AA | 18 (19.1%) | 7 (7.4%) | 33 (35.1%) | 30 (31.9%) | 6 (6.4%) |
| AT | 7 (33.3%) | 1 (4.8%) | 8 (38.1%) | 5 (23.8%) | 0 (0%) |
| TT | 1(100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| rs1142345 | TT | 22 (22%) | 7 (7%) | 34 (34%) | 31 (31%) | 6 (6%) |
| TC | 3 (21.4%) | 1 (7.1%) | 6 (42.9%) | 4 (28.6%) | 0 (0%) |
| CC | 1(100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| rs1800460 | CC | 24 (22.6%) | 7 (6.6%) | 37 (34.9%) | 32 (30.2%) | 6 (5.7%) |
| CT | 1 (11.1%) | 1 (11.1%) | 4 (44.4%) | 3 (33.3%) | 0 (0%) |
| TT | 1(100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| *ACYP2* |  |
|  |  | **Grade 0****(n=31)** | **Grade 1a****(n=17)** | **Grade 1b****(n=15)** | **Grade 2a (n=5)** | **Grade2b****(n=10)** | **Grade 3 (n=32)** | **Grade 4** **(n=5)** |
| rs1872328 | GG | 29 (27.4%) | 16 (15.1%) | 10 (9.4%) | 5 (4.7%) | 10 (9.4%) | 31 (29.2%) | 5 (4.7%) |
| GA | 1 (16.7%) | 1 (16.7%) | 4 (66.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| AA | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |

**Table 3: Multivariable regression analysis of the association between *COMT and TPMT* polymorphisms and cisplatin-induced ototoxicity using CTCAE grading as an ordinal outcome**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Estimate (SE) | Overall P-value | Bonferroni corrected P- value |
| *COMT* rs9332377 (CC) |  |  |
| *Grade 1 vs Grade 0:**Grade 2 vs Grade 0:**Grade 3 vs Grade 0:* *Grade 4 vs Grade 0:* | 1.03 (0.72)0.19 (0.53)-0.54 (0.60)0.47 (0.76) | 0.30 | 1.00 |
| *COMT* rs4646316 (CC) |
| *Grade 1 vs Grade 0:**Grade 2 vs Grade 0:**Grade 3 vs Grade 0:* *Grade 4 vs Grade 0:* | 0.52 (0.77)-0.10 (0.56)0.079 (0.59)-0.24 (0.94) | 0.93 | 1.00 |
| *TPMT* rs12201199 (AA) |
| *Grade 1 vs Grade 0:**Grade 2 vs Grade 0:**Grade 3 vs Grade 0:* *Grade 4 vs Grade 0:* | -1.07 (1.12)-0.92 (0.65)-1.66 (0.78)-17.6 (2940) | 0.068 | 0.34 |
| *TPMT* rs1142345 (TT) |
|  *Grade 1 vs Grade 0:**Grade 2 vs Grade 0:**Grade 3 vs Grade 0:*  *Grade 4 vs Grade 0:* | -0.47 (1.10)-0.43 (0.70)-0.72 (0.83)-16.9 (3450) | 0.55 | 1.00 |
| TPMT rs1800460 (CC) |
| *Grade 1 vs Grade 0:**Grade 2 vs Grade 0:**Grade 3 vs Grade 0:*  *Grade 4 vs Grade 0:* | -0.041 (1.10)-0.13 (0.78)-0.23 (0.96)-16.4 (3990) | 0.83 | 1.00 |

## Analysis undertaken using worse ear grade (in cases of asymmetric hearing loss)

**Table 4: Multivariable regression analysis of the association with the *ACYP2* genetic variant using worse ear grade (Chang classification) and ordinal outcomes**

|  |  |  |
| --- | --- | --- |
| *ACYP2* rs1872328 (GG) | Estimate (SE) | Overall P-value |
| *Grade 1a vs Grade 0:**Grade 1b vs Grade 0:**Grade 2a vs Grade 0:* *Grade 2b vs Grade 0:* *Grade 3 vs Grade 0:* *Grade 4 vs Grade 0:* | 0.70 (1.55)2.60 (1.32)-15.5 (6790)-16.8 (7650)-17.6 (4950)-16.4 (7360) | 0.027 |

**Table 5: Multivariable regression analysis of the association with the *ACYP2* genetic variant in Caucasians using worse ear grade (Chang classification) and ordinal outcomes**

|  |  |  |
| --- | --- | --- |
| *ACYP2* rs1872328 (GG) | Estimate (SE) | Overall P-value |
| *Grade 1a vs Grade 0:**Grade 1b vs Grade 0:**Grade 2a vs Grade 0:* *Grade 2b vs Grade 0:* *Grade 3 vs Grade 0:* *Grade 4 vs Grade 0:* | -18.1 (10086)1.50 (1.59)-17.3 (9704)-17.8 (10123)-18.3 (9122)-16.9 (9971) | 0.29 |

|  |
| --- |
| Table 6: Key information about the papers included in the systematic review |
|  | **Number of patients** | **Study design** | **Ethnicity** | **SNP(s) investigated** | **Outcomes and Definitions** |
| Hagleitner et al 2014 |
| Dutch cohort | 110 | Retrospective  | Dutch | *TPMT* rs12201199, rs1800460 and rs1142345; *COMT* rs4646316 and rs9332377 | Hearing loss, CTCAE version 3.0 (Grade 0 vs 2-4) and SIOP Boston ototoxicity scale (Grade 0 vs 2-4). Unclear whether worse or better ear. |
| Spanish cohort | 38 | Retrospective  | European |
| Lanvers-Kaminsky et al 2014 |
|  | 63 | Retrospective | unclear | *TPMT* rs12201199 and *COMT* rs9332377 | Hearing loss , Muenster classification (Grade 0 vs 2-4). |
| Pussegoda et al 2013 |
|  | 155 | Case-control | 80% Caucasian 20% other | *TPMT* rs12201199, rs1800460 and rs1142345; *COMT* rs4646316 and rs9332377 | Hearing loss, CTCAE version 3 criteria (Grade 0 vs 2-4). Unclear whether worse or better ear. |
| Ross et al 2009 |
| Discovery cohort | 53 | Case-control | 85% European 15% Non-European | Genotyped for 1,949 SNPs using customized genotyping assay designed to capture the genetic variation of 220 key drug metabolism genes | Ototoxicity , CTCAE version 3 criteria (Grade 2-4 vs 0). Unclear whether worse or better ear. |
| Replication cohort | 109 | Case-control |
| Yang et al 2013 |  |  |
| Cohort A | 213 | Retrospective  | 79% White 21% non-White | *TPMT* rs1800462, rs12201199, rs1800460 and rs1142345; *COMT* rs4818, rs4646316 and rs9332377 | Hearing loss, worse ear , CTCAE version 3 criteria (Grade 0 vs >0, Grade 0 vs 2-4 and analysed as ordinal variable) and Chang criteria, (Grade 0 vs Grade>0; Grade <2a vs ≥2a and analysed as ordinal variable) |
| Cohort B | 14 | Retrospective | 61% White 39% non-White |
| Xu et al 2015 |  |  |  |  |  |
| Discovery cohort | 238 | Retrospective | 66% European American, 34% Other. | GWAS 2,602,667 SNPs on Illumina HumanOmni2.5+HumanExome BeadChip. | Time to hearing loss (worse ear), classified as Chang score>0 and analysed as ordinal variable. |
| Validation cohort | 68 | Retrospective  | Unclear | *ACYP2* rs1872328; rs7604464. | Time to hearing loss (worse ear), classified as Chang score>0 |
| Vos et al 2016 |  |  |  |  |  |
|  | 156 | Retrospective  | 99% European | *ACYP2* SNP rs1872328 | Hearing loss , Chang score, Grade 0 vs Grade>0 |