Pharmacogenetics in patients with childhood acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL)

The most common pediatric cancer

• Therapy is decided by clinical feature of patients
  • Sex, age, genetic variant, etc...
• Survival has been dramatically improved and reached 90% in developed countries.

Goal of childhood ALL therapy: The best outcome with the least toxicities

Multiple drug therapy in childhood ALL

- Glucocorticoids
- Vincristine
- L-asparaginase
- Cytarabine
- Anthracyclines
- Cyclophosphamide
- 6-mercaptopurine
- Methotrexate
Effect of genetic variation

Therapy response factor: age, digestive tract function, diet, genetic variation...

Genetic variation affects protein expression

Drugs → activate → inactivate

Transporter expression change → Change pharmacokinetics

Germline variants

Drug tolerability in individuals

Somatic mutation

Response and resistance to therapy in cells

## Pharmacogenetics in childhood ALL

<table>
<thead>
<tr>
<th>Drug</th>
<th>Toxicity</th>
<th>Gene</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-mercaptopurine</td>
<td>Myelosuppression</td>
<td>TPMT, NUDT15</td>
<td>Mainly in Europe, Mainly in Asia</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>Hyper sensitivity</td>
<td>HLA-D region, GRIA1</td>
<td>USA, Hungary</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Neuropathy</td>
<td>CEP72</td>
<td>USA, Slovenia, Spain</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Low clearance</td>
<td>SLCO1B1</td>
<td>USA</td>
</tr>
</tbody>
</table>
6-mercaptopurine (6-MP)

Combination with methotrexate in maintenance (1–2 years) in childhood ALL
Genetic factors related to 6-MP

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT</td>
<td>Thioprine S-methyltransferase</td>
<td>Myelosuppression</td>
</tr>
<tr>
<td>ITPA</td>
<td>Inosine triphosphatase</td>
<td>Neutropenia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatotoxicities</td>
</tr>
<tr>
<td>ABCC4</td>
<td>ABC Transporter Subfamily C, Member 4</td>
<td>Myelosuppression</td>
</tr>
<tr>
<td>NUDT15</td>
<td>Nudix hydrolase 15</td>
<td>Myelosuppression</td>
</tr>
</tbody>
</table>

Therapy shortening owing to myelosuppression lead to risk of relapse

⇒ appropriate dose adjustment to avoid the severe toxicities
Thiopurine S-methyl transferase (TPMT)

Total 47 haplotype have been reported. (TPMT nomenclature committee)

Common inactivating alleles
c.238G>C, c.460G>A, and c.719A>G

MP, mercaptopurine; TGMP, thioguanine monophosphate; TGTP, thioguanine triphosphate

**TPMT** variant and toxicities

6-MP \(\rightarrow\) TGMP \(\rightarrow\) methyl TGMP

TPMT \(\rightarrow\) methylation

Incorporation to RNA \(\rightarrow\) severe myelosuppression

6-MP dose and metabolite level

Requiring dose reduction

**TPMT** low activity allele increase 6-MP metabolite level and induced severe toxicities.

### TPMT variant and outcome

<table>
<thead>
<tr>
<th>Trial</th>
<th>6-MP adjustment by TPMT</th>
<th>Outcome in TPMT low activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJCRH Total XII</td>
<td>No</td>
<td>TPMT$^{LA}$ higher risk of secondary AML</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TPMT$^{LA}$ higher cumulative risk of brain tumor</td>
</tr>
<tr>
<td>NOPHO-ALL-92</td>
<td>No</td>
<td>TPMT$^{HA}$ related higher rate of relapse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TPMT$^{LA}$ related higher rate of SMN</td>
</tr>
<tr>
<td>SJCRH Total XIBB</td>
<td>Yes</td>
<td>TPMT activity not related to relapse</td>
</tr>
<tr>
<td>NOPH-ALL-2000</td>
<td>Yes</td>
<td>Similar risk of relapse in TPMT status</td>
</tr>
<tr>
<td>UK-ALL-2003</td>
<td>TPMT deficiency</td>
<td>EFS and OS were not different in TPMT genotype</td>
</tr>
</tbody>
</table>

6-MP dose adjustment by TPMT genotype

- Reduce therapy related SMN and obtain similar outcome in variant
The frequency of poor metabolizer was rare in the Asian population (<0.1%).

The frequency of low TPMT activity is rare in Asians, and limited efficacy in 6-MP initial dose adjustment.
**TPMT** low activity in 6-MP therapy

- *TPMT* low activity leads to increase TGNs concentration and the risk of severe myelosuppression in 6-MP therapy

- Appropriate dose adjustment by *TPMT* variant improve outcome

In Asian population

- The frequency of *TPMT* low activity is lower compared to other races
- The effect of *TPMT* genotyping for adjustment therapy is small effect
6-MP tolerability in race

6-MP tolerability is affected by other than *TPMT* variants

**NUDT15**

Patients with Crohn’s disease in Korea

Strong association with early leukopenia

Patients with childhood ALL

NUDT15 and TPMT associated with 6-MP dose intensity


NUDT15 in thiopurine therapy

6-MP → thioGMP → thioGDP → thioGTP → RNA
NUDT15

thioGTP and thio dGTP concentration
Thiopurine induced severe leukopenia


**NUDT15 R139C**

in Japanese childhood ALL patients

<table>
<thead>
<tr>
<th>NUDT15 R139C (415C&gt;T)</th>
<th>CC (N=68)</th>
<th>CT (N=18)</th>
<th>TT (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukopenia (WBC&lt; 2 x 10⁹/L)</td>
<td>20 (29.4%)</td>
<td>13 (72.2%)</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>Leukopenia &lt; 60 days</td>
<td>12 (17.6%)</td>
<td>5 (27.8%)</td>
<td>5 (83.3%)</td>
</tr>
</tbody>
</table>

- 6-MP dose was required to be reduced to 8 mg/m².
- The EFS for this variant was tend to inferior to that for the wild-type.
**NUDT15 haplotype**

19 haplotypes have been reported in the PharmVar database. 8 haplotypes have shown low NUDT15 activity.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>PharmVar</th>
<th>CPIC guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>Normal function</td>
<td>Normal function</td>
</tr>
<tr>
<td>*2</td>
<td>Severe decreased</td>
<td>No function</td>
</tr>
<tr>
<td>*3</td>
<td>Severe decreased</td>
<td>No function</td>
</tr>
<tr>
<td>*4</td>
<td>Severe decreased</td>
<td>Uncertain function</td>
</tr>
<tr>
<td>*5</td>
<td>Severe decreased</td>
<td>Uncertain function</td>
</tr>
<tr>
<td>*6</td>
<td>Severe decreased</td>
<td>Uncertain function</td>
</tr>
<tr>
<td>*7</td>
<td>Severe decreased</td>
<td>Uncertain function</td>
</tr>
<tr>
<td>*8</td>
<td>Severe decreased</td>
<td>Uncertain function</td>
</tr>
<tr>
<td>*9</td>
<td>Severe decreased</td>
<td>Uncertain function</td>
</tr>
</tbody>
</table>

*17*
## Diplotype and NUDT15 activity

<table>
<thead>
<tr>
<th>NUDT15 activity</th>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal metabolizer</td>
<td>*1/*1</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>*2/*2, *2/*3, *3/*3, *4–*9</td>
</tr>
</tbody>
</table>

*4–*9 are very low frequencies and have been designated as unclear function.
The frequency of poor metabolizer was more than 10% in the Asian population. NUDT15 poor metabolizer in Asia is more frequently than TPMT poor metabolizer in Caucasian.
*1/*5 and *1/*6 were tolerable similar dose to wild-type (*1/*1).
**NUDT15 low activity genotype and clinical status**

Total of 17 patients treated with TCCSG protocol or JCCG protocol

Standard 6-MP dose were 40 to 50 mg/m²/day/p.o.
These dose were adjusted to maintain white blood cell counts of 2.0–3.5 x 10⁹/L

<table>
<thead>
<tr>
<th>NUDT15 genotype</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>*2/*2</td>
<td>1</td>
</tr>
<tr>
<td>*2/*3</td>
<td>5</td>
</tr>
<tr>
<td>*3/*3</td>
<td>8</td>
</tr>
<tr>
<td>*2/*5</td>
<td>1</td>
</tr>
<tr>
<td>*3/*5</td>
<td>1</td>
</tr>
<tr>
<td>*5/*5</td>
<td>1</td>
</tr>
</tbody>
</table>

Tanaka Y et al. ASH 2018 poster presentation
Eleven patients (64%) experienced 6-MP-induced severe myelosuppression during early consolidation therapy.

**Cumulative 6-MP-induced toxicities**

- Leukopenia Grade 4
- Neutropenia Grade 4
- ALT elevation Grade 3

**Averate 6-MP dose**

- Initial 6-MP dose (mg/m²) 28.3 (2–50)
- Average 6-MP dose (mg/m²) 7.0 (2.7–18.3)
- Therapy interruption 16 (94%)
- Duration of interruption (day) 66 (5–376)

Tanaka Y et al. ASH 2018 poster presentation
6-MP therapy in characteristic cases

**Case 1**

NUDT15 \(*2/3\)

T-ALL, 10-year-old boy

Ave. 6-MP dose was 13.1 mg/m\(^2\)

Interruption period was 255 days (35%)

**Case 2**

NUDT15 \(*2/3\)

BCP-ALL, 3-year-old girl

Ave. 6-MP dose was 3.7 mg/m\(^2\)

Interruption period was of 5 days (1.1%)

**Case 3**

NUDT15 \(*5/5\)

BCP-ALL, 8-year-old boy

Ave. 6-MP dose was 13.7 mg/m\(^2\)

Interruption was not experienced.

Tanaka Y et al. ASH 2018 poster presentation
Therapy outcome in NUDT15 low activity genotype

- 4-year OS 78%
- 4-year EFS 76%

Cause of death (2 patients)
- Severe infection after transplantation at the 3rd relapse
- 2nd malignancy

Tanaka Y et al. ASH 2018 poster presentation
NUDT15 variant for 6-MP therapy

- The *NUDT15* variant increase TGNs (thioGTP and thio dGTP) level, and increase the incorporating ratio of TGNs into DNA and RNA.

- NUDT15 low activity is risk of early severe myelosuppression.

- The effect of therapeutic outcomes has been uncleared

- The frequency of NUDT15 poor metabolizer is >10%, and *NUDT15* genotype is possible candidate factors of pharmacogenetics in Asia
CPIC guideline for thiopurine in 2018

TPMT and NUDT15 genotyping

TPMT normal

TPMT intermediate (IM)

TPMT poor

NUDT15

Normal

IM

Poor

Dose adjustment by NUDT15

Dose adjustment by TPMT

Dose adjustment less than 1 variant

Zhou et al. BMC Cancer 2018
# Recommended 6-MP by *NUDT15* genotype

<table>
<thead>
<tr>
<th>NUDT15 Phenotype*</th>
<th>Dosing recommendation for 6-MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal metabolizer (wild-type)</td>
<td>Normal initial dose</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>Protocol dose ≥75 mg/m²</td>
</tr>
<tr>
<td>Possible intermediate metabolizer</td>
<td>30%-80% of the normal dose</td>
</tr>
<tr>
<td>Protocol dose &lt;75 mg/m²</td>
<td>Not necessary to reduce the initial dose</td>
</tr>
<tr>
<td>Poor metabolizer (Homozygous)</td>
<td>10 mg/m² for malignancy</td>
</tr>
<tr>
<td></td>
<td>Consider alternative therapy for immunosuppressant therapy</td>
</tr>
</tbody>
</table>

*The phenotypes are defined as *NUDT15* diplootype

Possible intermediate metabolizer:
Carrying one uncertain function and one non-function allele


**NUDT15 and ABCC4 variants**

Genotyping \( ABCC4 \) and \( NUDT15 \) to prevent 6-MP tolerability

![Graph showing the cumulative incidence of WBC < 2.0 x 10^9/L and the course of 6-MP dose.](image)

- **P = 2.48 x 10^{-7}**
- **Both NUDT15 & ABCC4**

- **NUDT15 intermediate**
- **ABCC4 variant**
- **Wild type**

- **Average 6-MP dose (mg/m^2)**

- **The course of 6-MP dose**

Somatic mutation

**NT5C2**

- ALL tumor cells
- Combination chemotherapy
- NT5C2 mutated tumor cell
- DNA synthesis
- Cellular export and detoxification
- Tumor cell survival
- NT5C2 mutated
- Toxic metabolite
- Tumor cell killing

**Relapse** (19% patients)

**PRPS1**

- 6-MP
- NT5C2
- [^{13}C_6-^{15}N_2]-hypoxanthine
- HPRT1
- TPMT
- Salvage pathway
- PRPP
- PRPS1 (24/380)
- Competitive inhibition
- [^{13}C_6]-glucose
- DNA damage response
- Apoptosis

**Aster JC and DeAngelo DJ. Nat Med 2013; 19: 264-5**


**NT5C2 mutation**  →  Thiopurine resistant

**PRPS1 mutation**  →  relapse
Pharmacogenetics in childhood ALL patients

<table>
<thead>
<tr>
<th>Drug</th>
<th>Toxicity</th>
<th>Gene</th>
<th>East Asian</th>
<th>European</th>
<th>African</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparaginase</td>
<td>Allergy</td>
<td>GRIA1 rs4958351</td>
<td>3%</td>
<td>36%</td>
<td>25%</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Neuropathy</td>
<td>CEP72 s924607</td>
<td>31%</td>
<td>41%</td>
<td>8%</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Low clearance</td>
<td>SLCO1B1 rs11045879</td>
<td>45%</td>
<td>19%</td>
<td>19%</td>
</tr>
</tbody>
</table>

The validation results of these factors has been reported limitedly in Asian.

The main genetic variant influencing clinical status might be different from each race.
Conclusions

*NUDT15* variant is a strong pharmacogenetic factor for predicting 6-MP tolerability in Asian childhood ALL patients.

Some patients have unknown 6-MP intolerable factors.

We have accumulated Asian experiences internationally, and propose additional recommendations in Asian ALL therapy.